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A LABORATORY MANUAL

OF

PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY.

FOR STUDENTS IN MEDICINE

BY

DR. E. SALKOWSKI

Professor in the University and Director of the Chemical Laboratory of the Pathological Institute, Berlin

AUTHORIZED TRANSLATION FROM THE SECOND REVISED AND ENLARGED GERMAN EDITION

BY

W. R. ORNDORFF, A.B., PH.D.

Professor of Organic and Physiological Chemistry in Cornell University

With Ten Figures and a Colored Plate of Absorption Spectra

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FIRST THOUSAND

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DEDICATED TO

Professor Rudolf Virchow

AS A TOKEN OF THE ESTEEM AND GRATITUDE OF

THE AUTHOR.

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PREFACE TO THE ENGLISH EDITION.

I AM very much pleased that Dr. Orndorff has taken upon himself the translation into English of the second edition of my book "Practicum der physiologischen und pathologischen Chemie." The exact knowledge of German which Dr. Orndorff has acquired by long residence in Germany, together with his well-known scientific qualifications, guarantees a correct translation.

That part of the book dealing with inorganic chemistry has not been translated, as the students in medicine in America have usually completed a required course in qualitative analysis before they take up organic and physiological chemistry. In the description, moreover, of the Kjeldahl method of determining the amount of nitrogen in organic substances, the modification adopted by the "Association of Official Agricultural Chemists of the United States," which is the one generally used in America, has been given. It has therefore also seemed expedient to omit the Schneider-Seegen method.

A number of errors in the German edition have been corrected in this translation; and some additions and changes have been made, those due to Dr. Orndorff being indicated in foot-notes. The tables of specific gravities given are the most accurate that have been published.

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I cherish the hope that the book in this new form will contribute its modest share towards gaining new friends abroad for the study of physiological chemistry, so successfully pursued in America.

DR. E. SALKOWSKI.

BERLIN, Dec. 19, 1902.

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ABBREVIATIONS.

cm. for centimeter.	cc. for cubic centimeter.
mm. for millimeter.	g. for gram.
l. for liter.	mg. for milligram.

kg. for kilogram.

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A LABORATORY MANUAL

OF

PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY.

CHAPTER I.

EXAMINATION OF MILK.

I. General Properties of Milk.
II. Separation of Milk into its Constituents.
III. Precipitation with Magnesium Sulphate.
IV. Action of Rennin on Milk.
V. Preparation of Lactic Acid.

I. GENERAL PROPERTIES.

1. **The reaction of milk** is usually amphoteric, i.e., it reddens blue litmus paper, turns red litmus paper blue, and does not act on violet litmus paper. Towards phenol-phthaleïn it reacts acid, towards lacmoid alkaline.

2. **On heating fresh milk to boiling,**[1] it does not coagulate, its general appearance is not changed. When heated for a longer time it forms a skin on the surface consisting essen-

[1] All reactions or tests are to be performed in test-tubes unless otherwise directed.

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tially of evaporated milk. The specific odor of milk becomes stronger on heating. Milk which is a few days old coagulates on heating.

3. **On the addition of acids,** milk coagulates, the casein being precipitated.

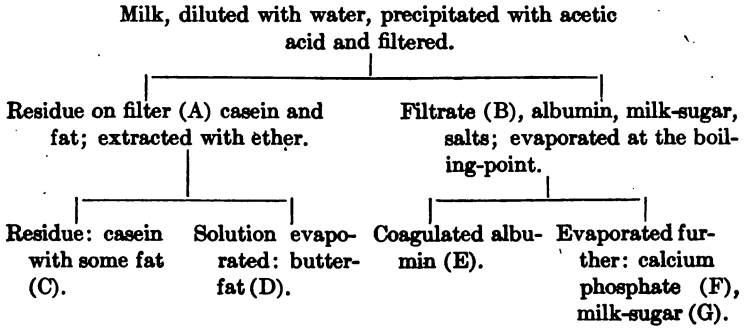
4. When equal volumes of milk and **caustic soda** solution are heated to boiling, the mixture becomes yellow and finally brown, owing to the action of the caustic soda on the milk-sugar.

5. If we shake a few cubic centimeters of milk with one and one-half to two times its volume of **ether,** the appearance of the milk is only slightly changed, though the fat is for the most part extracted. In order to show this, pour a part of the ether into a watch-glass, taking care that none of the milk gets into the glass. On evaporating in the air the ether leaves the fat behind. If we now pour some caustic soda solution down the side of the tube and mix the contents by shaking gently, the milk becomes almost clear. This shows that the white color of milk is due only in small part to the fat which it contains; to a much greater extent it is due to the swollen casein (and calcium phosphate).

6. When some tincture of **guaiacum** (made by dissolving some gum guaiacum in alcohol in a test-tube) is added to a little milk, then some old oil of turpentine, and the mixture shaken, it becomes blue. The color appears first at the surface of contact of the milk and the oil of turpentine (Kowalewsky). Boiled milk does not show this reaction. Milk, blood or blood-pigment, and the pus-cells possess this property in common. To distinguish boiled milk from unboiled, the following method of Schaffer is also recommended: add to 10 cc. of milk one drop of a 0.2 per cent. solution of hydrogen peroxide and two drops of a 2 per cent. solution of paraphenylene diamine. Fresh milk turns blue, boiled milk does not.

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II. Separation of Milk into its Constituents.



Dilute 400 cc. of milk (whole milk) in a beaker with 1 liter of water, and then add acetic acid,[1] cautiously with stirring, until the casein separates in coarse flakes. An excess of acetic acid is to be carefully avoided. The operation may be made easier by taking out small amounts of the milk in a beaker, adding acetic acid, and observing whether the casein becomes more coarsely flocculent. The casein incloses the fat completely and carries it down with it. The action of the acetic acid is to remove the alkali by means of which the casein is dissolved in the milk. The mixture is filtered through muslin, or the clear supernatant fluid is siphoned off and only the residue filtered.

The mixture (A) of casein and fat remaining on the filter is washed once with water, drained, and the filter is lightly squeezed with the hand to remove the excess of wash-water. It is then ground in a mortar with 100 cc. of absolute alcohol, which takes up the greater part of the water and some of the fat, and, after standing for half an hour, filtered. The alcohol is evaporated in a porcelain dish on the water-bath and the dish with the residue set aside. To separate the

[1] Thirty per cent. acid, sp. gr. 1.041, is always understood by this term. See table of reagents at the end of the book.

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casein and fat put the casein containing the fat into a dry flask with about 100 cc. of ether, shake thoroughly, allow to stand twenty-four hours, and then filter.

The casein remaining on the filter (C) is washed once more with ether, then pressed between filter-paper, and ground in a mortar until the casein, still containing some fat, forms a white dry powder.

The ethereal solution (D) is poured into the evaporating-dish in which the alcohol extract was evaporated and the solution allowed to evaporate spontaneously, taking care that the ether vapor does not come into contact with a flame. Finally it is separated from a small residue of water, alcohol, and ether by evaporating on the water-bath. Thus we obtain the butter-fat.

The fluid (B) separated from the casein is filtered through paper and then evaporated about one-half at the boiling-point in a tinned vessel or in an enamelled-iron dish. The albumin (E) precipitates in coarse white flakes; it is filtered off and washed a few times with hot water. The filtrate from the albumin is evaporated further over a free flame until it begins to bump. The bumping is due to the separation of calcium phosphate (F). Filter once more and evaporate on the water-bath; the sirupy solution yields an abundant crop of milk-sugar crystals when allowed to stand till next day.

Properties and Reactions of the Constituents of Milk.

1. Coagulated Albumin (E).

General Reactions of the Coagulated Albumins.

1. Xanthoproteic Reaction. A portion of the substance the size of a pea is heated in a test-tube with concentrated nitric acid (sp. gr. 1.2). The albumin turns yellow and at the same time a yellow solution is formed. When perfectly cold supersaturate with caustic soda solution. The color turns to

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orange and the undissolved particles of albumin also take on the same color. This reaction depends upon the conversion of the aromatic group in the albumin molecule into nitro derivatives.

2. **Millon's Reaction.** Treat a small portion of the substance with a few cubic centimeters of water, add some some Millon's reagent, and heat to boiling. The albumin turns brick-red. This reaction is due to the tyrosin group present in the albumin molecule; tyrosin gives the reaction in a striking manner, and the same is true of all derivatives of benzene in which a benzene hydrogen atom is replaced by hydroxyl. (O. Nasse.)

3. **Conduct towards an Alkaline Solution of Lead Hydroxide.** To some cubic centimeters of caustic soda solution add two drops of a neutral lead acetate solution. The precipitate of lead hydroxide first formed dissolves on shaking. Heat a small portion of the albumin with this alkaline solution of lead hydroxide; the mixture turns black in consequence of the formation of lead sulphide. A part of the sulphur is present in albumin in the unoxidized form and may be split off by alkalies as potassium or sodium sulphides.

4. **Reaction of Adamkiewicz.** Grind a part of the albumin in a small mortar with some absolute alcohol, filter, squeeze out the excess of alcohol, and grind with some cubic centimeters of ether, filter, and remove the excess of ether by pressure. Use half of the albumin thus obtained for the reaction of Adamkiewicz; dissolve the albumin by warming with a few cubic centimeters of glacial acetic acid, cool the solution, (by dipping into water), and allow some concentrated sulphuric acid to flow slowly down the wall of the test-tube into the acetic acid solution of the albumin: the fluids mix at the surface of contact, giving a violet to purple color.

According to Hopkins and Cole [1] this reaction is due to

[1] Proc. Roy. Soc., 68, 21 (1901).

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the presence of glyoxylic acid in the glacial acetic acid. If the reaction does not take place, add a small amount of a solution of oxalic acid which has been previously treated with sodium amalgam.

5. **Liebermann's Reaction.** Add to the other half of the albumin, treated with alcohol and ether, a few cubic centimeters of fuming hydrochloric acid and warm: bluish to blue solution, which, on standing, becomes paler or more violet or brownish. Sometimes only the violet color appears.

6. **Detection of Nitrogen.** A small portion of the albumin, which has been previously treated with alcohol and ether and then dried, is mixed with five to ten times its volume of soda-lime and the mixture heated in a narrow dry test-tube. Ammonia is given off (odor; alkaline reaction of the gas; fumes which it forms when brought into contact with a glass rod moistened with hydrochloric acid; blackening of a piece of filter-paper moistened with a solution of mercurous nitrate.)

The test for nitrogen with soda-lime fails in the case of certain forms of combination of nitrogen, e.g., with the nitro compounds. Of more general application and also more delicate is the **Lassaigne Test.** Heat the substance with a small piece of potassium (or sodium) in a narrow test-tube. A violent reaction takes place. After the test-tube has cooled somewhat, dip it into 10 cc. of water contained in a small beaker; the test-tube breaks and its contents dissolve in the water. When nitrogen is present the solution will contain potassium cyanide. Filter the solution, add a drop of ferric chloride solution and a few drops of ferrous sulphate solution to the filtrate and warm. The potassium cyanide forms potassium ferrocyanide. Cool the solution and acidify with hydrochloric acid. Green or blue color or a blue precipitate due to the formation of Prussian blue.

7. **Detection of Sulphur.** 1. Heat a very small piece of albumin in a narrow hard-glass test-tube, the upper part of

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which contains a narrow strip of filter-paper moistened with a solution of basic lead acetate; blackening due to the formation of lead sulphide (Siegfried). 2. Grind together 0.1 to 0.2 g. of the substance with thirty times its weight of oxidizing mixture (3 parts of potassium nitrate and 1 part of sodium carbonate), and heat the mixture slowly in a crucible or small dish until it is completely fused and all the carbon has been burned. The sulphur is oxidized to sulphuric acid, which forms alkali sulphate. After cooling dissolve the fused mass by warming with some water, filter in case the solution is not perfectly clear, acidify with hydrochloric acid, and add barium chloride solution. If the substance contained sulphur, a precipitate of barium sulphate will separate at once, or after some time. Since the test depends upon the conversion of sulphur into sulphuric acid, the substance and the reagents must of course contain no sulphates.

This method is not adapted to detect traces of sulphur: in this case we must evaporate the solution of the fused mass to dryness several times with hydrochloric acid in order to drive out the nitric acid. If we then add water to the residue, we sometimes find the solution is cloudy, due to the presence of silicic acid. It must then be filtered again, since clearness of the solution is absolutely essential. The sulphur may also be detected by heating to red heat with sodium (or potassium), sodium sulphide being formed. In many cases it is sufficient tc heat with sodium carbonate. See in this connection the "Detection of Sodium Sulphide" in the chapter on "Bile," section "Taurin."

2. Calcium Phosphate (F).

The calcium phosphate is washed with water, then dissolved by pouring on the filter 20 cc. of dilute hydrochloric acid (1 part hydrochloric acid, 2 parts water). The filtrate is frequently somewhat cloudy, but it may be rendered clear by allowing it to stand and by filtering several times, or also by repeatedly pouring it back upon the filter. The greater

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part of the filtrate is made alkaline with ammonia, acidified with acetic acid and then divided into two parts; to one part, in order to prove the presence of calcium, add ammonium oxalate (white precipitate of calcium oxalate); to the other part add uranyl nitrate (yellowish-white precipitate of uranyl phosphate). The phosphoric acid may also be tested for directly in the hydrochloric acid solution by means of ammonium molybdate. Add a few drops of the hydrochloric acid solution to some cubic centimeters of the ammonium molybdate solution: yellow precipitate.

3. Casein (C).

In order to purify the casein and especially to free it from the fat, put it into a dish with 250 cc. of water and add very dilute caustic soda solution (1 : 10) with constant stirring and quite slowly. The mixture must at no time have a strong alkaline reaction. When the greater part of the casein has dissolved, filter. The filtrate is usually somewhat cloudy. If necessary it may be again filtered; absolute clearness of the filtrate is, however, only attained with difficulty. The solution is precipitated by acidifying cautiously with acetic acid. The casein which separates is first washed by decantation, which may usually be done without any essential loss, then filtered and washed.

This is used in the following reactions:

1. Since casein is essentially a proteid, it gives all the reactions of the coagulated and insoluble proteids described under albumin.

2. A portion is shaken with water and a few drops of sodium carbonate solution; it dissolves therein clear or almost clear. If the solution is quite cloudy (fat, calcium phosphate), then the casein must be redissolved in water containing sodium hydroxide and be precipitated once more with acetic acid.

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3. A portion is ground with water and some calcium carbonate, and filtered. The filtrate, which is usually not quite clear, contains casein, as may be shown by acidifying with acetic acid. Casein therefore has the character of an acid: it drives out the carbonic acid and forms a soluble salt with calcium.

4. With a portion of the substance try the reaction with the alkaline solution of lead hydroxide described under Albumin. Only a faint gray color results. Casein contains only a little of the unoxidized sulphur; the greater part is in the oxidized form. In order to show more exactly the difference between casein and albumin in this reaction, we proceed as follows: the solution of caustic soda containing the lead is diluted with several times its volume of water and the dilution is continued until some of the diluted solution when boiled with albumin is only slightly blackened. The casein is then tested with this solution. It should react negatively.

5. Casein is not a simple proteid, but may be split up by appropriate means (digestion in the stomach) into a proteid and paranuclein. Since paranuclein, like nuclein itself, contains organically combined phosphorus, the casein also contains phosphorus. In order to prove the presence of phosphorus, we grind about 0.2 g. of the casein, which has previously been treated with alcohol and ether, with 6 g. of the oxidizing mixture (see above under Detection of Sulphur), heat to fusion, dissolve the fused mass, after cooling, in nitric acid and heat the solution in order to drive out the nitrous acid formed. A part of the solution is added, drop by drop, to about 5 cc. of the molybdate solution; yellow color, cloudiness, and then a yellow precipitate prove the presence of phosphoric acid, formed from the phosphorus by the fusion with the niter. The reaction is only to be relied on to prove the presence of phosphorus when the substance

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is free from calcium or magnesium phosphate.[1] We determine this by adding ammonia to the rest of the solution. It must remain clear.

4. Butter-fat (D).

The butter-fat is saponified. All heating and evaporating in this section is to be done on the water-bath. Put 5 g. of caustic potash into a flask, add 5 cc. of water, and dissolve the potash by warming. Then melt the butter-fat and pour it into the flask. Wash the dish with about 50 cc. of 90 per cent. alcohol, add the alcohol to the flask, and heat the mixture, with constant shaking, until it becomes homogeneous. The fat is thus split up into fatty acids and glycerin, saponified. In order to determine whether the saponification is complete, pour a small portion of the mixture into a little water; it must form a clear solution or one which becomes clear on gently warming. If it does not do this the mixture must be again heated. When the saponification is completed pour the contents of the flask into an evaporating-dish, drive off the alcohol by heating (water-bath), and when cold acidify with 30 cc. of dilute sulphuric acid. The fatty acids separate in the form of an oil, while at the same time the odor of butyric acid becomes perceptible, due to the presence of butyrin in the milk-fat. This butyrin is characteristic of milk-fat. It is only found in this substance.

The more detailed investigation of fat and the fatty acids will be taken up in the chapter on "Subcutaneous Adipose Tissue."

5. Milk-sugar, $C_{12}H_{22}O_{11}+H_2O$.

The milk-sugar (G) is separated from the mother-liquor by draining and pressing between filter-paper and is recrys-

[1] Alkali phosphates could not be present owing to the previous treatment.

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tallized from hot water. For this purpose put the milk-sugar into a flask, dissolve it by heating with water (some calcium phosphate always remains undissolved), add a little bone-black to decolorize, and filter hot. Evaporate the solution to about 25 or 30 cc. (to sirupy consistency) on the water-bath, let stand till next day, and place the crystals which have separated on drying-paper.

The milk-sugar forms hard, shining crystals, which dissolve in six parts of cold water, more readily in hot water, and only slightly in alcohol.

REACTIONS OF MILK-SUGAR.

1. A small quantity heated on a crucible-cover or on platinum-foil turns brown, gives off the odor of caramel, carbonizes, and finally burns completely, leaving very little ash.

Milk-sugar like grape-sugar is oxidized in alkaline solution; a number of sugar reactions depend upon this property. For the following reactions use a solution of 2 g. in 100 cc. of water and another solution 10 times as dilute (10 cc. of the solution diluted to 100 cc.).

2. **Trommer's Test.** To a few cubic centimeters of the solution add half the volume of caustic soda solution (of about 1.17 sp. gr.), then add copper sulphate solution, drop by drop, shaking the tube after the addition of each drop. A deep-blue solution results, which on heating gives a precipitate of red cuprous oxide (or yellow cuprous hydroxide). This property of dissolving cupric hydroxide in alkaline solution is common to milk-sugar, glucose, and to many other organic substances, such as cane-sugar, glycerin, mannite, and tartaric acid; but on warming these solutions no reduction takes place, except in the case of milk-sugar and glucose.

3. **Moore's Test.** On the addition of an equal volume of caustic soda solution of specific gravity 1.34 and heating to

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boiling, the solution turns yellow, then brown, and develops the odor of caramel, especially after acidifying with dilute sulphuric acid.

4. **Bismuth Test.** Saturate some of the solution at the boiling-point with solid sodium carbonate, add a little bismuth subnitrate, heat to boiling, and keep boiling for some time: gray or black color due to the formation of finely divided metallic bismuth. This reaction may also be performed with sodium hydroxide instead of sodium carbonate, using only a very slight amount of caustic soda, but the simultaneous action of the alkali on the milk-sugar cannot be excluded and the color is then a dirty grayish green.

5. To a few cubic centimeters of the solution add some sodium carbonate solution and a little freshly prepared potassium ferricyanide solution. Decolorization on warming, due to the formation of ferrocyanide of potassium.

6. To some of the solution add silver nitrate solution and ammonia and warm. Separation of metallic silver in the form of a bright mirror or a gray powder. A very pretty mirror is obtained if we use an excess of caustic soda with only a little ammonia in the reaction (caution on account of the possible formation of fulminating silver). Cane-sugar and mannite also give the reaction under these conditions, but not with ammonia and silver nitrate.

7. **Indigo Test.** Use only the weaker solution of the milk-sugar in this test. To a small portion of the sugar solution add some freshly prepared solution of indigo carmine or sodium indigo sulphonate until a blue color is produced, make alkaline with a few drops of sodium carbonate solution, and warm. The solution turns first violet, then red, then yellow, and finally becomes almost colorless. The reaction depends on the reduction of indigo blue to indigo white. Pour half of the solution into another test-tube and shake thoroughly with air. It turns blue again: oxidation of the

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indigo white to indigo blue. Heat again and the solution will be again decolorized. This process may be repeated until all the sugar is used up by oxidation.

All these reactions are given by glucose as well as by milk-sugar. The conduct towards yeast is the simplest method of distinguishing between the two sugars. Glucose is very quickly converted into alcohol and carbon dioxide by yeast, whereas milk-sugar is not, or at least very slowly and incompletely. In order to carry out the experiment shake a quantity of the 2 per cent. milk-sugar solution in a test-tube with a piece of compressed yeast as large as a hazel-nut, fill a fermentation-tube with the mixture (mercury seal), and put the tube in a warm place (about 35°). Make a similar test with a 2 per cent. solution of glucose as a check. After some hours the glucose solution will be found in fermentation, as shown by the development of carbon dioxide, which fills a part of the tube; the milk-sugar solution does not ferment. It is advisable to set up a third tube, which contains only water and yeast. No fermentation should take place in it during twelve to twenty-four hours. Gradually a slight development of carbon dioxide makes itself apparent (spontaneous fermentation of yeast).

The following tests may also be used to distinguish between the two sugars.

1. **Rubner's Test.** Dissolve 4 g. of neutral lead acetate by warming with about 5 cc. of the 2 per cent. solution of milk-sugar, boil for one to two minutes, then add an excess of ammonia and heat again. A deep-red solution will be formed and gradually a precipitate of the same color appears if sufficient ammonia has been added. Glucose acts in the same way at first, but the color soon becomes yellow (chamois).

Perhaps the following slight modification of the test is somewhat simpler: mix about 3 cc. of milk-sugar solution (2 per cent.) with the same volume of basic lead acetate solution and 1 cc. of ammonia, and boil for some time. The milky fluid becomes first yellow, then brick-red, and then remains unchanged, further addition of ammonia in-

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ceasing the color. Glucose solution (2 per cent.), when treated in the same way, though it does not become milky at first, also yields a red color and more quickly than the milk-sugar; the precipitate, however, soon becomes yellow. If more ammonia be added (2 cc.) there will be formed at first a very beautiful cherry-red color, which, however, also disappears very quickly.

2. **The Formation of Mucic Acid, $C_6H_{10}O_8$.** Five grams of milk-sugar are put into a flask with 15 cc. of nitric acid of specific gravity 1.2, and 5 cc. of nitric acid of about 1.48 specific gravity,[1] and the mixture is cautiously heated until the beginning of a violent reaction (strong evolution of oxides of nitrogen). Remove the flame at once and let stand till next day. Pour off the nitric acid from the crystallized mucic acid, wash with water, first by decantation, then on the filter, and let it dry on filter-paper. To identify the substance dissolve a portion of the mucic acid in an excess of ammonia, evaporate the solution to dryness on the water-bath, and heat the dry residue in a dry test-tube: pyrrol, C_4H_4NH, is formed. The vapor colors a pine splinter, moistened with strong hydrochloric acid and held in the mouth of the tube, a deep-red. If we treat glucose in the same way with nitric acid nothing separates. The solution contains a considerable quantity of oxalic acid, but no mucic acid.

3. **The Conduct of Milk-sugar on Heating with Hydrochloric Acid.** Dissolve about 6 g. of commercially pure milk-sugar in 125 cc. of water by heating to boiling, let cool, and determine how much this solution rotates the plane of polarized light. To 100 cc. of this solution in a flask add 10 cc. of hydrochloric acid and heat for half an hour; nearly neutralize the solution with dilute caustic soda (complete neutralization would make the color of the solution too dark), let cool, pour into a 100-cc. measuring-flask or into a measuring-cylinder, fill up to 100 cc., mix thoroughly, and again determine the amount of rotation. It will be found to have increased (hydrolysis of the milk-sugar into glucose and galactose, which latter rotates more to the right than lactose). When glucose is treated in the same way its rotation remains unchanged or decreases a little, in consequence of the formation of humin substances.

[1] Or, instead of the mixture, 20 cc. of nitric acid, 1.3 specific gravity.

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III. PRECIPITATION WITH MAGNESIUM SULPHATE.

Precipitate: casein (sodium) and fat.	Filtrate: milk-sugar, albumin, salts.

Casein may also be precipitated from milk, presumably in combination with alkali, in a form soluble in water or dilute salt solutions. The precipitation of the casein in this form results from the saturation of the milk with different salts, e.g., magnesium sulphate. Saturate 100 cc. of milk with magnesium sulphate (about 50 g. are required) by shaking it with the finely powdered salt in a flask. When the salt is entirely or almost entirely dissolved, filter through a filter moistened with a saturated solution of magnesium sulphate, and wash several times with magnesium sulphate solution. The precipitate contains casein and fat besides a globulin-like body (lactoglobulin) present in milk in very small quantity. In the filtrate we may prove the presence of albumin by heating to boiling. The well-washed and still moist precipitate of casein and fat is ground in a mortar with 100 cc. of water, when the casein goes into solution. The mixture is allowed to stand till next day, when the fat will have come to the top; it is then clarified by filtering several times. We thus obtain a light-bluish opalescent solution, scarcely ever entirely clear, which, however, may be used to determine the amount of rotation. The solution is lævorotatory. On the addition of acetic acid the casein is precipitated.

IV. ACTION OF RENNIN ON MILK.

(*a*) **Coagulation of Milk.** One-tenth (0.1) gram of the commercial rennet powder is dissolved in 100 cc. of water. The solution is usually somewhat cloudy, but it may be used

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without filtering.[1] 100 to 200 cc. of milk[2] are heated in a beaker to 40°, 5–10 cc. of the rennin solution added and well stirred. Coagulation soon takes place. The coagulum, consisting of casein and fat, gradually separates, leaving a fluid containing albumin and sugar (milk-serum or sweet whey). The process is perfectly analogous to the coagulation of blood with the formation of the blood-clot and separation of the blood-serum. The casein differs somewhat in its properties from the casein precipitated by acids. We therefore give it the name **paracasein** or **cheese.** Let stand till next day, then pour off as much of the fluid as possible, grind the coagulum, the cheese, with water, filter, wash again with water, and press the cheese dry between folds of muslin. In order to remove most of the fat we proceed in the same manner as given under Casein. When washed a few times with ether the paracasein is almost free from fat.

The paracasein like the casein dissolves readily in lime-water as well as in water to which sodium hydroxide or sodium carbonate solution has been added. It is reprecipitated on the addition of acetic acid. When ground with water and calcium carbonate the paracasein does not dissolve as readily as the casein. The paracasein always contains calcium; soluble calcium salts are essential for the coagulation of milk by rennin (Hammarsten). The sweet whey gives the guaiacum-turpentine reaction, and when heated it forms a voluminous precipitate of albumin.

If we add to 100 cc. of milk 5 cc. of a 1 per cent. solution of sodium oxalate, then the rennin ferment and heat to 40°, the milk does not coagulate on account of the precipitation of the calcium as calcium oxalate. If, however, we add a

[1] The solution appears to be more active when twenty-four hours old than when freshly prepared.

[2] Preferably skimmed milk.

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small quantity of calcium chloride, coagulation takes place at once.[1]

(*b*) **Influence of Acids and Alkalies on the Coagulation of Milk by Rennin.** Into each of three test-tubes A, B, and C put 10 cc. of milk. To B add 10 drops of dilute hydrochloric acid (1 cc. of hydrochloric acid, sp. gr. 1.183, to 150 cc. of water). No separation of casein should take place. To C add 1 to 2 drops of a concentrated solution of sodium carbonate. To A add neither acid nor alkali. To each of the three tubes add one-half a cubic centimeter or 10 drops of the rennin solution and note the order in which the coagulation takes place. The milk in B coagulates first, then that in A. The milk in C does not coagulate at all or coagulates extremely slowly. Acids aid the rennin coagulation; alkalies interfere with it or prevent it entirely. The coagulation of milk in the stomach results from the simultaneous action of the hydrochloric acid and the rennin ferment. The experiment may also be performed in another way. Dilute the rennin solution so that one-half of a cubic centimeter or 10 drops of it will just bring about the coagulation of 100 cc. of milk in ten minutes or will no longer coagulate it. Now add to 10 cc. of milk 10 drops of the dilute hydrochloric acid (0.25 per cent.) and then 10 drops of the diluted rennin solution. Coagulation results before the expiration of ten minutes.

V. LACTIC ACID FERMENTATION.[2]

To a solution of 50 g. of cane-sugar in 500 cc. of water add 20 g. of precipitated chalk and about 30 cc. of sour milk, and let the mixture stand, in an open flask or one loosely

[1] Arthus and Pagès, Arch. de Physiologie, 1891, pp. 331 and 540.

[2] For a better method of preparing lactic acid see Die Zersetzung stickstofffreier organischen Substanzen durch Bacterien von Dr. O. Emmerling, p. 32.—O.

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stoppered with cotton, six to eight days at a temperature of 40°, shaking the flask frequently. The cane-sugar will be transformed for the most part into lactic acid, which combines with the calcium. After the lapse of the time given most of the chalk will be dissolved. Boil the mixture, filter, concentrate to a small volume on the water-bath, and allow to crystallize. The crystallized calcium lactate is dried by pressing between drying-paper and purified by recrystallizing from hot water, using some bone-black to decolorize if necessary. The calcium salt is then converted into the zinc salt. Determine the weight of the air-dried calcium salt and weigh off the equivalent quantity of zinc sulphate (to 308 parts of calcium lactate 287 parts of the crystallized zinc sulphate) or a little less. Dissolve each salt in a little water, mix the solutions, filter, after allowing to stand for some time, from the calcium sulphate which separates, evaporate on the water-bath, let stand till next day, and purify the zinc lactate crystals obtained by recrystallization (microscopic crystal form). From the zinc lactate we obtain the free lactic acid by conducting hydrogen sulphide through its solution until the zinc is completely precipitated. The solution filtered from the zinc sulphide is evaporated and the lactic acid obtained is purified by dissolving in a mixture of equal volumes of alcohol and ether (small quantity), filtering and evaporating.

The fermentation lactic acid, $CH_3CHOHCOOH$ (ordinary, inactive, ethylidene lactic acid), forms a colorless or pale-yellow sirupy fluid of strong acid reaction, miscible in every proportion with water, alcohol, and ether.

For the lactic acid reaction of Uffelmann see the chapter on Digestion.

A part of the lactic acid obtained is used for the preparation of pure zinc lactate. Boil with water and an excess of basic zinc carbonate, filter, and evaporate to crystallization (it is best to use the

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freshly precipitated basic zinc carbonate made as follows: 2 g. of zinc sulphate are dissolved in about 100 cc. of water, heated, sodium carbonate solution gradually added until the reaction is markedly alkaline, and the precipitate washed free from sulphates and sodium carbonate with hot water, first by decantation and then on the filter). The well-pressed air-dried zinc lactate, $(C_3H_5O_3)_2Zn+3H_2O$, contains 3 molecules of water of crystallization (18.17 per cent.), which is driven off at 100° to 110°. The determination of the amount of water of crystallization serves to distinguish it from the zinc sarcolactate, which only contains 2 molecules of water (12.89 per cent.). Drying over sulphuric acid instead of in the air is inadmissible, since a part of the water of crystallization is lost. Indeed by drying for fourteen days over sulphuric acid the whole of the water may be given off.

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CHAPTER II.

EXAMINATION OF MUSCULAR TISSUE.

I. Preparation of Creatine, Detection of the Xanthine Bases.
II. Detection of the Proteids.
III. Preparation of the Xanthine Bases or Alloxuric Bases.
IV. Preparation of Sarcolactic Acid.

I. PREPARATION OF CREATINE—DETECTION OF THE XANTHINE BASES.

Meat, finely ground, digested with water, filtered, and the residue subjected to pressure.

- Filtrate heated to boiling and filtered.
 - Residue.
- Filtrate precipitated with basic lead acetate and filtered.
 - Residue: coagulated albumin.
- Filtrate freed from lead by means of H_2S, filtered, and the filtrate evaporated: creatine.
 - Precipitate: lead phosphate, chloride, and sulphate.

Four hundred grams of finely ground meat,[1] as free as possible from fat and gristle, are thoroughly mixed with 800 cc. of water in a large dish and heated on a water-bath. Dip a thermometer into the mixture. It should show a temperature of 50° to 55°. After 20 minutes to half an hour, filter

[1] Beef or rabbit's flesh (the latter is very rich in creatine and especially suitable), or also dog's flesh. Horse-flesh is not suitable, as the extracts after the precipitation with basic lead acetate do not give a clear filtrate, presumably on account of the greater amount of glycogen in horse-flesh.

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through muslin and press out the residue completely in a press.

Heat the combined extracts to boiling in a thin-walled tinned vessel, stirring constantly, in order to precipitate the albumin. The fluid surrounding the coagulum of albumin must be quite clear. If it is not clear add a few drops of acetic acid. Filter from the albumin, which is colored somewhat red from the blood-pigment mixed with it, and let cool completely. The extract, entirely free from albumin, is cautiously treated with basic lead acetate solution as long as a precipitate forms, then filtered, the filtrate freed from lead [1] by means of hydrogen sulphide and again filtered. Test a portion of the filtrate to see if it is free from lead. When hydrogen sulphide is passed through it, no blackening should occur; if it does, then the filtrate must be again treated with hydrogen sulphide. The filtrate is now evaporated, on the water-bath, to the consistency of a thin sirup, and this is allowed to stand for some days in a cool place. The creatine crystallizes out. It is separated from the mother-liquor by filtering, if it separates in large crystals, or, if it crystallizes in small crystals, by pouring the whole mass on a porous plate. It is then recrystallized from a little hot water.

Creatine, $C_4H_9N_3O_2+H_2O$, forms transparent, colorless, hard, rhombic prisms, which easily lose their water of crystallization. It dissolves in 74 parts of cold water, more readily in hot, very slightly in alcohol, and not at all in ether. The solutions react neutral. Creatine has no characteristic reactions. For its recognition we make use of either its conduct when heated cautiously or its conversion into creatinine.

1. A small portion of the creatine is cautiously heated

[1] Instead of this we may also, in order to save time, precipitate the greater part of the lead by the cautious addition of sulphuric acid and then separate the remainder by means of hydrogen sulphide; however, the lead sulphate passes readily through the filter.

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on a crucible cover or a piece of platinum foil over a very small flame. It first loses its water of crystallization and becomes white like porcelain, then it turns brown, giving off a characteristic odor, carbonizes, and finally burns without leaving any residue—if pure.

2. **Conversion into Creatinine.** Heat the remainder of the creatine for half an hour with 10 cc. of dilute sulphuric acid (20 per cent.) on the water-bath, adding water to replace that lost by evaporation. To get rid of the sulphuric acid grind the solution, after the addition of water, in a mortar with barium carbonate until the mixture no longer reacts acid, filter, and evaporate the filtrate on the water-bath to a few cubic centimeters.

(*a*) **Creatinine Zinc Chloride,** $(C_4H_7N_3O)_2ZnCl_2$. In a watch-glass add to a few drops of the solution thus obtained a drop of an alcoholic solution of zinc chloride. A pulverulent or microcrystalline precipitate of creatinine zinc chloride soon separates. Examine under the microscope.

(*b*) **Weyl's Reaction.** Mix the greater part of the solution with sodium nitroprusside solution (freshly prepared by dissolving a few crystals in a little water) until the solution has a yellow color, then add a few drops of caustic soda solution. The fluid turns deep red to ruby-red, the color soon fades and becomes straw-yellow. If the solution is now acidified with glacial acetic acid (about one-fourth the volume) and heated to boiling or allowed to stand for some time it turns green, and deposits on longer standing a precipitate of Prussian blue.

The mother-liquor from the creatine contains the xanthine bases or alloxuric bases (earlier also called xanthine bodies), which may be obtained in the form of the silver compounds by adding ammonia to alkaline reaction, then filtering and adding an ammoniacal silver nitrate solution (see below).

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The residue of meat remaining after pressing is broken up, heated to boiling with water in a tin dish, and the water together with the fat floating on it is poured off. This operation is repeated once more and then the mass is filtered through muslin. The residue may be used for digestion experiments.

II. Detection of the Principal Proteids in Meat.

Meat extracted with cold water.

Filtrate contains soluble proteids.	Residue may be used for the preparation of myosin.

Mix 100 g. of finely ground meat with 300 cc. of water, stir thoroughly, let stand one to two hours, pour the mixture through a muslin filter and squeeze out the residue with the hand. The filtrate is colored red (from the hæmoglobin) and is usually somewhat cloudy on account of the fat and small particles of muscle. In order to make it clear it is filtered through paper.

1. **Filtrate.**

(*a*) Testing the Reaction. Tested with sensitive litmus paper the filtrate reacts acid in consequence of its containing primary potassium phosphate (KH_2PO_4). The acid reaction becomes stronger on keeping the meat on account of the formation of lactic and other acids.

(*b*) A portion of the filtrate is slowly heated in a test-tube in which a thermometer is placed. For this purpose put the test-tube in a beaker half filled with water and heat the beaker on the wire gauze. By frequently stirring the water with a glass rod, on the under end of which is a piece of rubber tubing, the even distribution of the temperature may be accomplished. Even at a slight elevation of temperature, usually at 55° to 56°, coagulation occurs, the fil-

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trate from the coagulum again coagulates at about 65°, and the filtrate from this at about 75°.

2. **Residue. Preparation of Myosin.** The meat residue is again extracted in the same manner with water, the water poured off, and then the residue stirred into a thin paste with a 15 per cent. solution of ammonium chloride, and after twenty-four hours filtered. The solution contains myosin.

(*a*) A part of the solution is poured into a test-tube two-thirds full of water. Separation of myosin in a swollen condition.

(*b*) A part of the solution is poured upon a piece of salt in a small beaker. The surface of the salt becomes covered with the precipitated myosin. Instead of this we may also cause the separation of the myosin by introducing finely pulverized salt into the solution and stirring.

(*c*) A portion of the solution is heated to boiling and filtered. The filtrate contains calcium salts, as may be shown by the addition of ammonium oxalate.

Myosin is therefore characterized by its insolubility in water and strong solutions of salts, solubility in salt solutions of medium concentration, and by the fact that it contains calcium, which it gives up on coagulation.

III. Preparation of the Xanthine Bases, Alloxuric Bases.

Method A.

Dissolve 50 g. of meat extract in 500 cc. of water in a flask and, after the addition of 75 to 100 cc. of nitric acid (1.2 sp. gr.) to destroy substances which hinder the precipitation of the xanthine bases by silver nitrate heat on the sand-bath until the solution has cleared up which will require about three-quarters of an hour. After cooling make strongly alkaline with ammonia, filter from the phosphates which

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separate, and add an ammoniacal solution of 2.5 g. of silver nitrate in about 100 cc. of water. The precipitate, which consists for the most part of hypoxanthine silver besides a little xanthine silver, is then collected on a filter and washed a few times with water.

Separation of Hypoxanthine and Xanthine.

The separation of these two xanthine bases is accomplished by converting them into the silver nitrate compounds. These compounds conduct themselves differently towards nitric acid. The hypoxanthine silver nitrate compound is very difficultly soluble in nitric acid; the xanthine silver nitrate compound is far more readily soluble. The following is the best method of procedure:

Put the still moist precipitate into a flask and pour over it a mixture of 100 cc. of nitric acid and 100 cc. of water, add 1 g. of urea, heat just to boiling, and let cool. The hypoxanthine silver contained in the precipitate is converted into hypoxanthine silver nitrate, which remains partly undissolved and partly goes into solution, but separates out of the solution again on cooling. The addition of the urea is to prevent the formation of nitrous acid, which might decompose the xanthine bases. Filter the hypoxanthine silver nitrate off after a few hours and wash until the wash-water no longer reacts strongly acid. Let the filtrate (without the wash-water) stand till next day and filter (without working up the precipitate, which is a mixture of hypoxanthine silver nitrate and xanthine silver nitrate). The filtrate is used to show the presence of xanthine. The hypoxanthine silver nitrate is examined under the microscope (fine needles frequently grouped in the form of stars).

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Conversion of the Hypoxanthine Silver Nitrate into Hypoxanthine.

(*a*) **By Means of Hydrochloric Acid.** Pierce the filter and wash the precipitate into a flask. Add a few cubic centimeters of hydrochloric acid, shake thoroughly and continuously, and finally warm gently. The hydrochloric acid decomposes the silver compound with the separation of silver



FIG. 1.—Hypoxanthine Silver Nitrate.

chloride. By thoroughly shaking and gently warming this settles well. The completion of the decomposition is controlled by a microscopical examination. Heating too strongly is to be avoided, as otherwise the aqua regia formed may destroy the hypoxanthine.[1] When the decomposition is complete, filter, make the filtrate alkaline with ammonia, and evaporate to dryness on the water-bath. Treat the residue with a small quantity of water, which dissolves the ammonium chloride and nitrate formed, but leaves the hypoxanthine undissolved.

[1] In order to avoid this it is advisable first to convert the hypoxanthine silver nitrate into the hypoxanthine silver compound (see below).

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(*b*) **By Means of Hydrogen Sulphide.** Put the hypoxanthine silver nitrate into a flask with some water and pass in hydrogen sulphide, shaking frequently, until the precipitate appears perfectly black and no white particles are to be seen. Filter: the filtrate contains the hypoxanthine nitrate. Concentrate somewhat on the water-bath in order to drive off the hydrogen sulphide, make faintly alkaline with ammonia, and proceed as above. The disadvantage of this method is that the complete decomposition with hydrogen sulphide is difficult to accomplish and that the hypoxanthine may contain some sulphur.

The decomposition may be accomplished more readily if the hypoxanthine silver nitrate is first converted into the silver compound of hypoxanthine by digesting it for some time with water, ammonia, and 2 g. of silver nitrate. Filter and wash the precipitate, suspend it in water, warm, and add ammonium sulphide, drop by drop. The silver sulphide settles on warming; the filtrate on evaporation yields hypoxanthine (Schindler [1]).

For the reactions the hypoxanthine obtained in any one of the above ways is sufficiently pure. In case it is markedly yellow it may be purified in the following manner: Dissolve the hypoxanthine in water by adding hydrochloric acid, add a few drops of ferrous sulphate, heat the solution and make it alkaline with sodium hydroxide, filter, concentrate somewhat, and precipitate the hypoxanthine by acidifying faintly with acetic acid or by neutralizing exactly with hydrochloric acid. Iron, in the form of ferrous hydroxide, often passes into the filtrate on filtering the alkaline solution. It is then necessary to let the filtrate stand for some time, shaking it frequently until the iron has completely separated as ferric hydroxide.

Method B.

In the method A, owing to the action of the nitric acid on the xanthine bases, so-called nitro compounds may easily be formed and contaminate the hypoxanthine. This may be avoided by getting rid of the substances, which interfere with precipitation of the xanthine bases, not by oxidation with nitric acid, but by precipitation. Dissolve 50 g. of meat extract in 500 cc. of water, add basic lead acetate to the

[1] Zeitschr. f. physiol. Chem., **13**, 433.

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solution as long as a precipitate is formed, filter, and remove the excess of lead from the filtrate with hydrogen sulphide. Boil the solution or concentrate it on the water-bath, in order to get rid of the excess of hydrogen sulphide, make strongly alkaline with ammonia, and precipitate with an ammoniacal solution of silver nitrate, etc., etc.

The hypoxanthine (or sarcine), $C_5H_4N_4O$, is very difficultly soluble in cold water (in 1400 parts at 19°), somewhat more soluble in hot water (70 parts). It dissolves readily in mineral acids, forming well-crystallized salts, and in alkalies, even in ammonia (distinction from guanine, which is but very slightly soluble in ammonia). It gives the so-called xanthine reaction, but less markedly than any of the other xanthine bases.[1]

Reactions of Hypoxanthine.

1. Pour upon a small portion of the substance on a porcelain crucible cover a few drops of strong or fuming nitric acid and evaporate cautiously to dryness over a small flame. A lemon-yellow residue results, which takes on an orange color when moistened, after cooling, with caustic soda solution. If a drop of water be then added, a yellow solution results, and this when evaporated again leaves an orange residue (distinction from the murexide reaction for uric acid).

2. Pour upon a small portion of the substance in a dish a little pure nitric acid of the specific gravity 1.2, and evaporate on the water-bath to dryness. The residue is scarcely perceptibly colored. On the addition of caustic soda it becomes pale yellow (distinction from xanthine and guanine, which under these conditions give the xanthine reaction). Hypoxanthine is further distinguished by its solubility in ammonia

[1] According to Hoppe-Seyler, Thierfelder, 7th edition, page 147, hypoxanthine does not give the xanthine reaction.—O.

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and the insolubility of its compound with silver nitrate in nitric acid, and also by the crystal form of this compound.

Detection of Xanthine.

The filtrate from hypoxanthine silver nitrate contains xanthine, as previously stated, but only in small quantity. Make it alkaline with ammonia (or, in order to save ammonia, neutralize the greater part of the acid with soda or lime and then make alkaline with ammonia). Xanthine silver precipitates in brown or reddish flakes. These are filtered off, washed, suspended in water, some drops of ammonia added, heated, treated with a few drops of ammonium sulphide, shaken thoroughly, filtered from the silver sulphide, and evaporated (or the precipitate may also be decomposed with hydrochloric acid and xanthine hydrochloride obtained on evaporation). Very frequently the silver sulphide passes through the filter; we then evaporate to dryness and extract the residue with boiling water. The xanthine thus obtained is usually not quite pure and the quantity is very small. It suffices, however, for the xanthine test as well as for the so-called Weidel's reaction.

Xanthine, $C_5H_4N_4O_2$, occurring very rarely in the form of stone in the bladder, results, like the hypoxanthine, from the decomposition of the nucleins (A. Kossel). In cold water it is practically insoluble (14,151 parts at 16°). In alcohol and ether it is insoluble. In hot water it is very difficultly soluble, but it is soluble in caustic soda and ammonia as well as in acids, forming salts.

1. **Xanthine Test.** Dissolve the residue or half of it in nitric acid and evaporate cautiously to dryness on a crucible cover over a small flame. A lemon-yellow residue results, which becomes intensely red on moistening with caustic soda, and on further heating purplish red. Add a few drops of water and warm; a yellow solution results, which again gives

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a red residue on evaporation (distinction from the murexide reaction for uric acid).

2. **The So-called Weidel's Reaction.**[1] Dissolve half of the xanthine obtained in bromine-water, warming gently, evaporate the solution on the water-bath to dryness, and invert the dish over another which contains some ammonia. The residue becomes red.[2]

IV. Preparation of Sarcolatic Acid.

For this purpose the filtrate from the silver precipitate III Method B may be used. Evaporate this to a sirup on the water-bath. Ammonia escapes and a portion of the silver separates in reduced form. Extract the residue with alcohol, filter the alcoholic extract, evaporate on the water-bath, dissolve in 75 cc. of water to which 25 cc. of dilute sulphuric acid have been added, and extract by shaking in a separating-funnel, three or four times at least, with one and a half volumes of ether to which a little alcohol has been added. Separate the ether, filter through a dry filter, and distil off the ether. It is advantageous to distil the first extract at once and use the distilled ether (adding some fresh ether) for the second extraction.

Use a small portion of the residue resulting for the Uffelmann's reaction for lactic acid (see chapter on Digestion). Dissolve the remainder in water, boil with freshly precipi-

[1] The best way to perform the test according to E Fischer (Ber. **30**, 2236 (1897) is to boil a small quantity of the finely powdered xanthine with some freshly prepared chlorine-water or with some hydrochloric acid and a little potassium chlorate, then cautiously evaporate the fluid to dryness on platinum foil and moisten the residue with ammonia (formation of murexide).—O.

[2] If we use, in addition to the bromine-water (originally chlorine-water was prescribed), a trace of nitric acid, the reaction is far more beautiful, but under these conditions other xanthine bases also give the test. This form of the reaction is therefore to be discarded as liable to lead to error.

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tated basic zinc carbonate, filter, evaporate to crystallization (examine microscopically), and when this has begun add alcohol. The zinc salt resulting is filtered off and freed from the mother-liquor clinging to it by pressing between filter-paper. If it is quite free from sulphuric acid [1] (after



FIG. 2.—Zinc Sarcolactate.

purification by recrystallization) make a determination of the amount of the water of crystallization. For this purpose weigh off on a watch-glass exactly 0.3 to 0.5 g. of the air-dried zinc salt, and heat for some time in an air-bath at 115° until the weight is constant. Zinc sarcolactate crystallizes with two molecules of water, $(C_3H_5O_3)_2Zn+2H_2O$ (12.89 per cent. H_2O). The loss in weight must therefore be 12.89 per cent.

Digestion with water containing chloroform is a very convenient method for the preparation of the xanthine bases. Five hundred grams of finely divided meat are placed in a glass-stoppered bottle, or divided between two bottles, with 5 liters of chloroform-water (5 cc. of chloroform to 1 liter; in this case ordinary water may be used), then a few drops more of chloroform are added, and the mixture is well shaken.

[1] The presence of the sulphuric acid may be avoided if phosphoric acid instead of sulphuric acid is used to acidify.

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Digest for two to three days at 40°, shaking repeatedly and thoroughly, then filter and drain the residue completely by pressure. Heat the extract in order to coagulate the albumin, evaporate the filtrate to about 500 cc., make strongly alkaline with ammonia, and precipitate with ammoniacal silver solution, etc. By means of the digestion the nuclein is decomposed and the substances which prevent the precipitation of the hypoxanthine silver are removed.

V. Detection of Organically Combined Phosphorus in Meat Extract.

According to Siegfried, meat extract free from albumin contains an organic substance (nucleon) in which phosphorus is present. Carniferrin is the name given by Siegfried to the iron compound of this substance. The following method may be used for the preparation of this compound: Dissolve 10 g. of meat extract in 200 cc. of water, make faintly alkaline with ammonia, add calcium chloride solution as long as a precipitate forms (the reaction must remain neutral during the precipitation and later a little more ammonia is to be added), and filter. To the filtrate add 15 cc. of a 3 per cent. ferric chloride solution, heat to boiling and, if necessary, neutralize exactly with ammonia. The precipitate is filtered off and washed. It dissolves in dilute sodium carbonate solution, forming a more or less clear solution. The remainder of the carniferrin is ground with alcohol, filtered, and then treated with a small quantity of ether. The presence of the phosphorus may be shown by fusing with the oxidizing mixture, dissolving the fused mass in water, filtering from the iron oxide, and testing for phosphoric acid in the filtrate.

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CHAPTER III.

GASTRIC DIGESTION.

I. Detection of Hydrochloric Acid in the Gastric Juice:
 (*a*) with methyl violet; (*b*) with tropæolin; (*c*) with Günsburg's reagent.
II. Detection of Lactic Acid (and Hydrochloric Acid):
 (*a*) by Uffelmann's method; (*b*) after extraction with ether.
III. Detection of Pepsin in Gastric Juice or Vomit.
IV. Influence of the Amount of Pepsin on the Intensity of Digestion.
V. Influence of Disturbing Substances, Qualitative.
VI. Comparison of Different Kinds of Pepsin.
VII. Preparation of the Products of Digestion.

I. DETECTION OF HYDROCHLORIC ACID IN THE GASTRIC JUICE.

Solutions required:

1. Six cubic centimeters of strong hydrochloric acid, specific gravity 1.19 (about 37 per cent. HCl), diluted to one liter: solution A. This solution contains 0.27 per cent. of HCl.

2. One hundred cubic centimeters of solution A diluted to 500 cc.: solution B.

3. Eight-tenths of a gram of lactic acid dissolved in 100 cc. of water.

4. Two grams of commercial peptone dissolved in 100 cc. of water.

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(*a*) Reactions with Methyl Violet (0.5 : 1000) or Gentian Violet.

1. A small portion of the hydrochloric acid solution A in a test-tube is treated with a few drops of methyl violet solution: steel-blue color. Make the same test with water: violet color. With the lactic acid solution: violet with a light-bluish tint.

2. **Influence of Dilution.** Repeat experiment I with the hydrochloric acid solution B.

3. **Influence of the Presence of Albumoses and Peptone.** Dilute some of the hydrochloric acid solution A with an equal volume of water, and another portion with an equal volume of the peptone solution, then add a few drops of the methyl violet solution to both and compare the colors. We may also perform the experiment by taking some of the steel-blue solution, produced by the action of the hydrochloric acid (A) on the methyl violet solution, dividing it into two parts, and adding the peptone solution to one part and water to the other part.

4. **Influence of the Peptone in very dilute Hydrochloric Acid.** Repeat experiment 3 with the hydrochloric acid solution B. From the results in 3 and 4 it will be seen that the methyl violet reaction cannot be used in the presence of considerable quantities of albumoses and peptone.

5. (*a*) To about 30 cc. of the lactic acid solution add some of the methyl violet solution and divide the mixture into three parts. To the first, A′, add an equal volume of water; to the second, B′, the same volume of water and then saturate the mixture with salt (NaCl); to the third, C′, the same volume of a 3 per cent. salt solution. A′ does not change its color, it only becomes somewhat clearer; C conducts itself in the same way; B′, however, becomes perceptibly steel-blue. Conclusion: sodium chloride in the presence of

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lactic acid interferes with the reaction only in quite concentrated solution, owing to the hydrochloric acid set free.

(*b*) Repeat experiment 5 (*a*), using a solution (0.25 g. to 1000 cc.) of tropæolin 00,[1] instead of the methyl violet.

The reactions may be made sharper by cautious evaporation of the mixtures (about 30 drops) in porcelain dishes.

(*c*) **Reactions with Günzburg's Reagent.** 1 g. vanillin, 2 g. phloroglucin, 100 cc. alcohol.[2]

1. Add a drop of Günzburg's reagent to a few drops of the hydrochloric acid solution A, evaporate to dryness in a small porcelain dish over a free flame. Avoid heating too strongly by moving the contents of the dish from side to side and blowing upon it during the heating: purple-red residue.

Repeat the experiment with the mixtures 2, 3, and 4. The peptone interferes with the Günzburg reaction less than with the previous ones. Lactic acid does not give the reaction.[3]

II. DETECTION OF LACTIC ACID.

(*a*) **With Uffelmann's Reagent.** To prepare this reagent add to 10 cc. of a 2 per cent. phenol solution a few drops of ferric chloride: amethyst-blue fluid.

[1] Only the tropæolin with this trade name is to be used.

[2] Günzburg's reagent cannot be kept long without undergoing change, and it works best when it is freshly made. The quantities do not make much difference, so that we can prepare the reagent by dissolving as much phloroglucin as may be held on the point of a small knife-blade and the same quantity of vanillin in a few cubic centimeters of alcohol in a test-tube.

[3] An alcoholic solution (0.2 per cent.) of dimethylamidoazobenzene is more sensitive towards free hydrochloric acid than Günzburg's reagent. The yellow solution is changed to red in the presence of free hydrochloric acid. Dilute solutions of the organic acids do not give the reaction. Filter-paper dipped into the above solution of dimethylamidoazobenzene and allowed to dry may also be used very advantageously to detect small quantities of free hydrochloric acid in the gastric contents. See J. Friedenwald, Medical Record, April 6, 1895.—O.

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1. Add a few drops of the reagent to some of the stronger hydrochloric acid solution A: decolorization.

2. Repeat with the lactic acid solution: lemon-yellow color.

3. Repeat with a mixture of equal parts of the hydrochloric acid solution A and lactic acid: lemon-yellow color, but fainter than in 2. Conclusion: lactic acid in the presence of hydrochloric acid may be detected by Uffelmann's reagent, but not hydrochloric acid in the presence of lactic acid.

4. Add to 15 cc. of the lactic acid solution some of Uffelmann's reagent and divide into three parts, A, B, and C. To A add its own volume of water, to B the same volume of a concentrated salt solution, to C the same volume of a 3 per cent. salt solution: only B is decolorized; the presence of sodium chloride, therefore does not as a rule interfere with the detection of lactic acid.

(*b*) **After Isolating the Lactic Acid by Means of Ether.** Mix 25 cc. of the hydrochloric acid solution A with 25 cc. of the lactic acid solution, shake with 50 cc. of ether, separate the ether and shake the aqueous fluid again with ether. Filter the united ether extracts through a dry filter and distil off the ether. Take up the residue in a little water and test the solution with Uffelmann's reagent. This method of isolating the lactic acid by means of ether is used especially with the fluids of the stomach when their color prevents or renders difficult the direct detection of the lactic acid.

III. Detection of Pepsin in the Gastric Juice or Vomit.

Add to 10 or 20 cc. of the fluid 10 to 20 drops of pure dilute hydrochloric acid (1 : 10), then add some shreds of fibrin or a slice of hard-boiled egg-albumen, and keep the mixture at 40°. The shreds of fibrin should dissolve even to

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the last particle in a quarter- to a half-hour, and the slice of egg-albumen should be perceptibly diminished after an hour if pepsin is present.

IV. Influence of the Quantity of Pepsin on Digestion.

Prepare an artificial digestive fluid from commercial pepsin and dilute hydrochloric acid (0.27 per cent.). If pepsin, which will dissolve in water forming a clear or almost clear solution, is to be had, dissolve 0.5 g. of it in 500 cc. of dilute hydrochloric acid (the hydrochloric acid used for digestion experiments is always a mixture of 6 cc. of strong hydrochloric acid, sp. gr. 1.19, with 994 cc. of water, i.e., 6 cc. of hydrochloric acid diluted to one liter: the solution designated on page 33 as hydrochloric acid solution A). If only the pepsin insoluble in water is available, which may nevertheless be very active, treat 0.5 g. of this with water, stir thoroughly, and wash with water until the filtrate no longer gives the reaction for milk-sugar. After piercing the filter, wash the residue into a flask with 100 cc. of the 0.27 per cent. hydrochloric acid, let stand with frequent agitation for twenty-four hours at the room temperature or at 40°, filter, and dilute with solution A to one-half of a liter.

Put into each of three test-tubes, A, B, and C, approximately equal quantities of fibrin,[1] preferably weighed quantities (about 1 g.). To A add 10 cc. of the dilute hydrochloric acid (0.27 per cent.), to B 5 cc. of the same hydrochloric acid and 5 cc. of the pepsin hydrochloric acid solution, to C 10 cc. of the pepsin hydrochloric acid, and place the tubes in a water-bath at 40°. The fibrin in A swells, but does not dissolve; in B and C it dissolves, and more quickly in C than in B. If the pepsin used is very active it may happen that no

[1] Instead of fresh fibrin we may also use here the fibrin which has been kept in glycerin, after it has been freed from the glycerin clinging to it by washing thoroughly with water.

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difference between B and C is perceptible; then more dilute solutions are to be used.

The experiment may also be performed by placing the fibrin and the dilute hydrochloric acid (0.27 per cent.) in all the tubes, adding nothing to A, to B 2 drops of a glycerin extract of a pig's stomach, and to C 4 drops of the same extract. According to Grützner the difference may be more readily perceived if we use fibrin colored with carmine. In order to color the fibrin let it lie for twenty-four hours in a 1 per cent. solution of carmine (rendered as nearly neutral as possible by evaporating the ammonia) and then wash the fibrin with water.

V. Interference of Certain Substances with Digestion.

Dissolve 2.5 g. of gum arabic, by shaking for some time, without heating, in 10 cc. of pepsin hydrochloric acid (A′). In an equal amount of the pepsin hydrochloric acid dissolve 5 g. of cane sugar (B′). A third portion is prepared without any addition (C′). In each of these tubes put 1 g. of fresh fibrin (or of the fibrin preserved in glycerin, after it has been well washed and drained), and digest at 40°. In C′ the fibrin dissolves quickly, slowly in B′, and still more slowly in A′. Many other indifferent substances, which have no affinity for hydrochloric acid, may also retard digestion.

VI. Comparison of the Different Kinds of Commercial Pepsin.

The method is somewhat different according as the pepsin is soluble in water or not. In the first case 1 g. is dissolved directly in 500 cc. of 0.27 per cent. hydrochloric acid. In the second case 1 g. is freed from milk-sugar as directed under IV, page 37, and then washed into a flask with 500 cc. of 0.27 per cent. hydrochloric acid (or it may also be treated directly, without any previous washing, with the 0.27 per cent. hydrochloric acid), digested in this for twenty-four hours at room temperature or at 40°, and then filtered through a

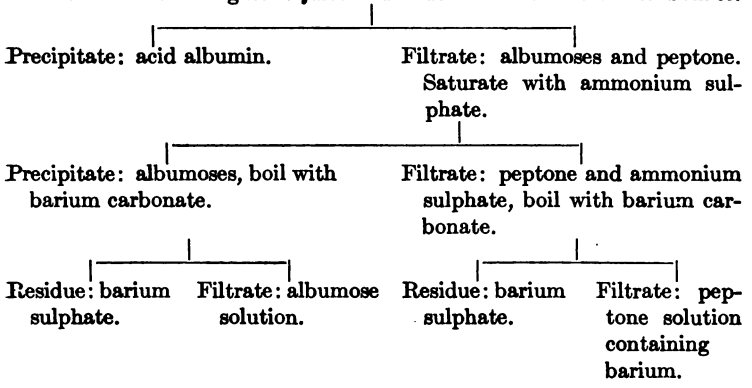
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dry filter. In a number of test-tubes digest equal weights of well-drained fibrin with 10 cc. of each of the above solutions of pepsin hydrochloric acid at 40°, and observe the difference in the time of digestion. In order to eliminate errors several tests should be made with each solution of pepsin hydrochloric acid. We use first 1 g. of fibrin; if no difference is perceptible we make a series of experiments with 0.5 g. and 2 g. Instead of the fibrin slices of egg-albumen may be used. The more exact determination can only be accomplished by means of quantitative analysis.

VII. Preparation of the Products of Digestion.

Digest 250 grams of fibrin for forty-eight hours at 40° with one and a half liters of artificial gastric juice and neutralize with sodium carbonate.



The best material for digestion is well-drained fresh blood-fibrin.

If only the coagulated fibrin preserved in chloroform-water is available, it is advisable first to subject it to the following treatment before using: Heat the fibrin in a large dish (enamelled-iron dish) with water containing 2 cc. of hydrochloric acid, specific gravity 1.19, to the liter, until it swells and becomes jelly-like. After cooling the jelly is ready for use. It dissolves almost as readily as the fresh fibrin. The swelling, however, sometimes remains incomplete, presumably when the fibrin was boiled too long before being put into the chloroform-water.

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If we desire to use the products of digestion for any experiments on animals, it is essential also that the fresh fibrin should be repeatedly extracted, first with water, and then with faintly acidulated water (not so much in order to cause it to swell as for the purpose of purifying it). If this is not done, the products of digestion may contain toxic substances (ptomaïnes or Gautier's leucomaïnes). For this extraction use water containing 1 cc. of hydrochloric acid, specific gravity 1.19, to the liter.

Instead of fibrin we may use for the digestion experiments the meat residue[1] obtained in the investigation of the soluble constituents of meat (see chapter on Muscular Tissue, page 23). Coagulated egg-albumen is also to be recommended as a very pure and satisfactory material. The albumen of a considerable number of eggs (about 20) is carefully separated from the yolks, beaten up in a cylinder with an equal volume of water, exactly neutralized with hydrochloric acid, filtered from the precipitate which forms (through paper or by pouring several times through muslin), the clear filtrate poured with constant stirring into boiling water, and the reaction ultimately made very faintly acid with acetic acid. Then heat to active boiling, filter, and wash the precipitate thoroughly with hot water.

When fibrin is used, digest it in a bottle or cylinder with at least five times the quantity of artificial gastric juice for forty-eight to seventy-two hours at 40°. Prepare the 1.5 liters of artificial gastric juice required as follows: Mix 3 g. of pepsin with water in a porcelain dish, filter, and wash until the filtrate no longer gives the reaction for milk-sugar. Then dilute 9 cc. of hydrochloric acid, specific gravity 1.19, to 1.5 liters with water. Pierce the filter containing the pepsin, wash the pepsin into a flask with a little water, and add 300 cc. of the dilute hydrochloric acid just made. Shake thoroughly, let stand at room temperature or at 40° until next

[1] In this case, however, the products of digestion will be contaminated with gelatin-albumose and gelatin-peptone.

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day, filter, and add the filtrate to the remaining 1200 cc. of the dilute hydrochloric acid.

When the meat residue from the 400 g. of meat is used about 2.5 liters of the artificial gastric juice are necessary; with the egg-albumen from 20 eggs, 2 liters. A glycerin extract of the stomach-lining (about 4 cc. of the extract to a liter of the dilute hydrochloric acid, 0.27 per cent.) is also much used for digestion experiments. Kühne[1] recommends to press out the contents of the rennet glands of a well-washed stomach by scraping with a spatula and to digest 10 g. of this paste with 1 liter of hydrochloric acid (containing 0.4 per cent. HCl) for four hours at 40° (Kühne's "normal gastric juice").

It is also recommended to extract the mucous membrane of the stomach directly with hydrochloric acid (0.4 per cent.). When pepsin hydrochloric acid is used the principal product is albumose with but little peptone. When the artificial gastric juice from the stomach-lining is used it is said that more peptone is formed. It may be remarked, however, that the action of the hydrochloric acid extract of the stomach-lining is not uniform and is often very deficient. This is due to the fact that this extract still contains slimy, unknown proteids, which greatly contaminate the products. To avoid these, Kühne[2] recommends a purified gastric juice made as follows: The prepared lining from the fundus of the pig's stomach is heated to 40° for six days with seven times the quantity of dilute hydrochloric acid containing 0.5 per cent. HCl. The solution resulting is then saturated directly with ammonium sulphate, when a voluminous, resinous precipitate forms. This is collected, freed from the salt solution as much as possible by pressure, washed quickly with water, and dissolved in dilute hydrochloric acid (0.5 per cent.) which contains 0.25 per cent. of thymol (using five times as much hydrochloric acid as of the stomach-lining originally taken). This solution is again heated for some days at 40° and then saturated with ammonium sulphate. The precipitate, "purified pepsin," is used for digestion experiments by suspending it in the 0.27 per cent. hydrochloric acid, using ten times as much of this dilute hydrochloric acid as of the stomach-lining originally taken.

When the digestion has continued for two to three days, the mixture being repeatedly stirred or shaken, filter the solution through muslin, heat gently, and neutralize it in a large

[1] Zeitschr. f. Biol. 19, 184. [2] Ibid. 22, 426, 428.

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dish with sodium carbonate solution, and filter from the precipitate formed. Show that the precipitate consists essentially of acid albumin (solubility in dilute alkalies, precipitation by mineral acids). The filtrate is evaporated, at first by heating it to boiling over a free flame. Some proteid, apparently globulin, always precipitates at this point. This is filtered off before the solution has been much concentrated. During the evaporation the reaction must be kept as nearly neutral as possible (by means of sodium carbonate or dilute hydrochloric acid). Evaporate on the water-bath to about 200 cc.[1] It is now necessary to separate the albumoses from the peptone. This may be done by saturating the solution, acidified with acetic acid, with sodium chloride, or by completely saturating it with ammonium sulphate. The albumoses are precipitated, while peptone remains in solution. The advantage of ammonium sulphate over acetic acid and salt lies in the more complete precipitation of the albumoses; however, even when ammonium sulphate is used some deuteroalbumose (Kühne) frequently remains unprecipitated; the disadvantage consists in the fact that the removal of the ammonium sulphate afterwards offers far greater difficulties than that of the sodium chloride.

Separation of the Albumoses from Peptone by Means of Ammonium Sulphate.

Put the solution evaporated to 200 cc. into a large mortar containing 100 g. of finely ground ammonium sulphate. Grind thoroughly until the ammonium sulphate is all dissolved and separate the viscous mass of albumoses, which precipitates, from the solution by decantation. The solution is kept; the precipitate is ground once more with a solution

[1] By precipitating a solution of this kind with alcohol the commercial peptone (mixture of albumoses and peptone) is prepared.

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of ammonium sulphate, separated from the solution, and this second solution then thrown away.

It is now necessary to free the albumose precipitate from the ammonium sulphate clinging to it and inclosed in it. For this purpose dissolve it in water and boil the tolerably dilute solution (best in an enamelled-iron dish) with barium carbonate, replacing the water which evaporates by hot water. By boiling with barium carbonate the sulphuric acid is combined with the barium and the ammonia escapes. Continue the boiling until the fluid no longer smells of ammonia and a filtered portion gives no cloudiness with barium chloride solution and therefore all the sulphuric acid is united to the barium. Then filter.[1] The albumose solution thus obtained very frequently, perhaps always, contains barium, often in considerable amount (shown by adding dilute sulphuric acid to a small portion of the solution). To separate the barium add ammonia and ammonium carbonate as long as a precipitate is formed, heat, and filter from the barium carbonate, best after allowing to stand for some time. Evaporate the filtrate on the water-bath to a small volume and precipitate with 95 per cent. or absolute alcohol. The albumoses precipitate in the form of a viscous mass. Let stand for some hours under the strong alcohol, best with renewal of the alcohol, until the mass has become hard and brittle, pour off the alcohol, grind the residue in a mortar with absolute alcohol, bring the whole mass into a vessel which may be closed, let stand in this for twenty-four hours, filter, and wash with ether. We thus obtain a fine white or yellowish-white powder, which according to Kühne is a mixture of four substances: dysalbumose, protalbumose, heteroalbumose, and deutero-

[1] The filtrate should be clear; however, a slight cloudiness does not matter in case the solution is afterwards to be treated with ammonium carbonate, as the precipitate of barium carbonate thereby formed carries down with it the remainder of the barium sulphate.

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albumose.[1] The separation of these bodies will not be taken up here. The first three of these albumoses are also called primary albumoses in contradistinction to deuteroalbumose.

(*a*) **Conduct of Albumose on Heating.** Heat a small portion for three hours to 130° to 140°, let cool, and treat with water. The albumose now only partly dissolves in water. Filter and wash. Warm the insoluble residue in a test-tube with dilute soda solution: partial solution. Acidify the filtered solution cautiously with hydrochloric acid: precipitate. The albumose is reconverted by heating into a body resembling albumin, presumably by the loss of water (R. Hofmeister).

(*b*) Dissolve 5 g. of the albumose by warming with 100 cc. of water. The solution formed is turbid (dysalbumose and residue of albumin). The filtered solution is used for the following reactions:

Reactions of Albumose.

1. Heat a small portion of the solution to boiling: it remains unchanged (after transient cloudiness) even after acidifying with acetic acid and adding a very few drops of sodium chloride solution.

2. Acidify with acetic acid and add some concentrated sodium chloride solution: the solution becomes cloudy, but clears up on heating. On cooling it again becomes cloudy.

3. Add to a small portion of the solution a few drops of nitric acid; a cloudiness or precipitate [2] soluble in an excess of the nitric acid results. The solution becomes lemon-yellow

[1] According to the investigations of R. Hofmeister and his pupils these substances are also in part not simple but mixtures.

[2] If the solution contains but little sodium chloride, the cloudiness may not appear. Repeat the experiment, in this case adding some salt solution before treating with the nitric acid. If the albumose is obtained from the meat residue, this reaction only takes place after the addition of sodium chloride.

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on standing or gently warming; on supersaturating with caustic soda this color changes to orange (xanthoproteic reaction).

4. Acidify the solution with a few drops of acetic acid and then add some potassium ferrocyanide solution: marked cloudiness which disappears on heating (often not completely).

5. Add to a portion of the solution half its volume of caustic soda and then, drop by drop, a dilute solution of copper sulphate. The copper hydroxide, which first precipitates, dissolves on shaking with a purplish-violet color: biuret reaction. An excess of copper sulphate changes the color of the solution to a blue violet. The biuret reaction is not characteristic of albumoses, as albumin also gives it.

A part of the albumose solution is then diluted to ten times its volume (0.5 per cent.).

1. **Biuret Reaction, Posner's Modification.** Float a dilute solution of copper sulphate on the diluted albumose solution, containing half its volume of sodium hydroxide solution (so that the copper sulphate solution runs down the wall of the tube, held in an inclined position, and the fluids do not mix). The characteristic color develops at the surface of contact of the two fluids or proceeds from this. Recommended for dilute solutions. With such solutions we may also use to advantage an ammoniacal solution of copper sulphate or Fehling's solution.

2. Add to small portions of the solution (1) mercuric chloride solution, (2) a solution of tannin, (3) some drops of hydrochloric and phosphotungstic acids: insoluble precipitates.

3. Add to a small portion of the solution a few drops of Millon's reagent and heat: red precipitate.

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Preparation of Peptone.

The solution obtained in the precipitation of the albumoses contains peptone together with traces of albumoses, especially deuteroalbumose. Dissolve, by heating, 20 g. more of ammonium sulphate in this solution, or so much of it as may be necessary to form a saturated solution, and make alkaline with ammonia and ammonium carbonate. After cooling, filter, heat until the odor of ammonia has disappeared, saturate again at the boiling-point with ammonium sulphate, let cool, and when perfectly cold filter from the ammonium sulphate and traces of albumose which separate.[1] Before preparing peptone from the filtrate determine by the biuret reaction whether it contains any considerable quantity of peptone. For this purpose add to a portion of the filtrate so much caustic soda solution of 1.34 specific gravity that sodium sulphate begins to separate, then add the copper sulphate solution. If an intensely red fluid does not result, further work with the solution will not pay. The removal of ammonium sulphate from the solution is accomplished in the same manner as with albumose, by boiling with barium carbonate, etc. It is advisable, however, before treating with barium carbonate to separate a part of the ammonium sulphate by evaporation and crystallization and also by precipitation with alcohol, in which the peptone dissolves. Since the barium carbonate as a rule is not entirely free from soluble salts, these together with the sodium chloride accumulate in the fluid containing the peptone, so that the peptone obtained always yields considerable ash.

The reactions are the same as those of albumose, but no

[1] To effect a complete separation, the solution made acid with acetic acid must be again saturated with ammonium sulphate at the boiling-point, allowed to cool, and filtered once more. Kühne, Zeitschr. f. Biol. **29**, 1 (1892).—O.

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precipitates are given by acetic acid and potassium ferrocyanide, acetic acid and sodium chloride or by nitric acid.

Instead of using ammonium sulphate we may also precipitate the albumoses from the acidified solution by means of sodium chloride. For this purpose add to the 200 cc. of the solution obtained 10 cc. of glacial acetic acid and grind the mixture with 75 g. of pure sodium chloride. The separation takes place the same as it did when ammonium sulphate was used: wash the albumoses with a concentrated solution of sodium chloride. The sodium chloride is removed by dialysis. After the dialysis concentrate the solution and precipitate with alcohol. We may also purify the precipitate by dissolving it in water, heating the solution, adding a solution of salt until the hot fluid is no longer clear, cooling and purifying the precipitate thus obtained by dialysis. The precipitation of the albumose by acetic acid and salt is not so complete as that with ammonium sulphate. The peptone remaining in solution therefore contains albumose.

According to S. Fränkel[1] albumose and peptone may be separated from each other by means of alcohol alone.

[1] Wiener. med. Blätter, 1896, Nos. 45 and 46.

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CHAPTER IV.

EXAMINATION OF BLOOD.

(*a*) Defibrinated Blood.

I. Alkaline Reaction of Blood.
II. Reaction with Guaiacum and Oil of Turpentine.
III. Conduct towards Hydrogen Peroxide.
IV. Solution of the Blood-corpuscles.
V. Crystallized Oxyhæmoglobin.
VI. Spectroscopic Examination of Oxyhæmoglobin, Hæmoglobin, Methæmoglobin, and Sulphohæmoglobin.
VII. Carbon Monoxide Hæmoglobin.
VIII. Alkaline Hæmatin Solution. Reduced Hæmatin.
IX. Hæmatin Hydrochloride.
X. Hæmin Test.
XI. Hæmatoporphyrin.
XII. Conduct of Blood on Heating.

(*b*) Blood-fibrin.

I. Conduct towards the 0.27 per cent. Hydrochloric Acid.
II. Conduct towards Hydrogen Peroxide.
III. Conduct towards Neutral Salts.

(*c*) Blood-serum.

I. Precipitation of the Albumin by Salts.
II. Separation of the Proteids.
III. Reactions of the Proteids of the Blood-serum.

(*a*) Defibrinated Blood.

I. Reaction of Blood.

The alkaline reaction of blood cannot be shown with ordinary litmus paper alone, since this becomes saturated with

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the blood-pigment or blood-corpuscles. But the reaction may be shown very beautifully if we allow a few drops of violet-red litmus tincture to soak into a porous clay plate, and then place upon this spot a drop of blood and wash it off at once. There is thus obtained a distinct, even intensely blue spot (Liebreich). But even with ordinary litmus paper the alkaline reaction may be shown in the following manner: Grind some blood in a mortar with an excess of powdered ammonium sulphate, so that even after long grinding a portion of the salt still remains undissolved. Into this paste dip a rather wide strip of red litmus paper and then wash it off thoroughly with water.—Zuntz recommends litmus paper made out of tissue-paper. Moisten this with a concentrated solution of salt or sodium sulphate or magnesium sulphate, place a small drop of blood on the paper with a glass rod, and absorb the liquid at once with blotting-paper.

II. Conduct towards Guaiacum and Oil of Turpentine.

To 8 or 10 cc. of water add a few drops of blood (shaking thoroughly), then some tincture of guaiacum (freshly prepared by dissolving some gum guaiacum in alcohol in a test-tube) till the solution becomes milky, and finally some oil of turpentine which has stood for some time. On shaking, the mixture becomes intensely blue (oxidation of the gum guaiacum: the blood-corpuscles act as carriers of oxygen from the ozonized oil of turpentine to the gum guaiacum).

III. Reaction with Hydrogen Peroxide.

To a few cubic centimeters of blood add several times the volume of a solution of hydrogen peroxide: marked frothing due to the escape of oxygen (so-called catalytic action of the blood-pigment).

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IV. Solution of the Blood-corpuscles.

Treat a small portion of the blood in a test-tube with some ether and some water, and shake thoroughly: the blood-corpuscles dissolve, the blood becomes transparent (laky blood). A solution of the salts of the bile-acids acts in the same way on the blood.

V. Preparation of Crystallized Oxyhæmoglobin.

The preparation of crystallized oxyhæmoglobin is readily accomplished only with certain kinds of blood (dog, horse, guinea-pig, rat), but not with the blood of man, ox, pig, or rabbit.

One hundred cubic centimeters of dog's blood are shaken vigorously in a flask with air, cooled to 0°, shaken with 10 cc. of ether and 10 cc. of water, so that the blood-corpuscles dissolve and the blood becomes laky (the complete solution of the blood-corpuscles is to be determined by means of a microscopical examination), and the mixture allowed to stand at 0°. The separation of the oxyhæmoglobin crystals takes place at once or after a few hours when dog's blood is used.

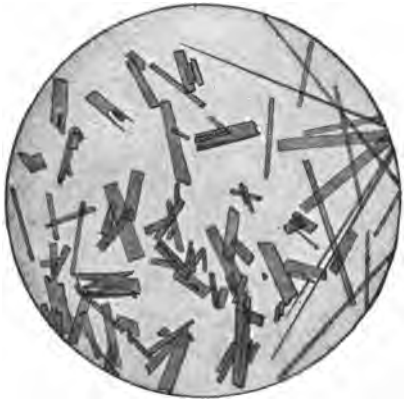


FIG. 3.—Crystals of Oxyhæmoglobin from Dog's Blood.

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Examine under the microscope. To purify the oxyhæmoglobin, filter at a low temperature, press out the mother-liquor, dissolve the crystals in as small a quantity of water as possible at 30°, filter quickly, add one-fifth to one-fourth the volume of alcohol gradually and with constant stirring to prevent coagulation, and let stand at 0° until crystallization is complete.

VI. Spectroscopic Examination.[1]

1. **Oxyhæmoglobin and Hæmoglobin.** Ten cubic centimeters of blood are diluted to 100 cc., filtered, then gradually further diluted until the solution when examined spectroscopically shows distinctly the oxyhæmoglobin bands between D and E in the yellow and green of the spectrum (see Table of Absorption Spectra,[2] No. 1). Then add to the solution a few drops of the so-called Stokes's solution (solution of ferrous ammonium tartrate, which must always be freshly prepared, made by dissolving in water a piece of ferrous sulphate as big as a pea, adding as much tartaric acid as may be held on the point of a knife-blade and then ammonia to alkaline reaction: clear greenish solution). The blood changes its color at once, becomes bluish or violet, and shows in place of the two absorption-bands a broad band due to hæmoglobin (No. 2 in the Table of Absorption Spectra). The reduction may also be brought about by adding a few drops of ammonium sulphide solution and allowing the mixture to stand for some minutes; but this takes longer and in addition to the broad band of the hæmoglobin there always appears a weaker and narrower band in the red, which presumably comes from the sulphohæmoglobin compound.

[1] For most work in physiological chemistry the Browning pocket spectroscope is to be preferred on account of its convenience.—O.

[2] For a more recent table of absorption spectra, see Ziemke and Müller, Archiv für Anat. u. Physiol., 1901, Sp. Bd. 177.—O.

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2. **Methæmoglobin.** To prepare a solution of methæmoglobin, add to some of the same or a more concentrated solution of blood a few drops of a strong solution of potassium ferricyanide (freshly prepared). The solution turns brown and shows a characteristic spectrum (No. 3 in the Table of Absorption Spectra). Especially to be noted is the strong absorption of light in the blue part of the spectrum. Now add to the methæmoglobin solution a few drops of ammonium sulphide, let stand some minutes, and then shake vigorously with air. The solution now shows the bands of oxyhæmoglobin. The methæmoglobin may therefore be converted by reduction and subsequent oxidation into oxyhæmoglobin.

3. **Sulphohæmoglobin.** Conduct hydrogen sulphide into the blood solution, which has been previously thoroughly shaken with air. It turns brown, then dirty green, due to the formation of sulphohæmoglobin, and when examined with the spectroscope shows an absorption-band between the yellow and orange near the C line (E. Harnack).

VII. Carbon Monoxide Hæmoglobin.

Conduct illuminating-gas (or carbon monoxide) into 50 cc. of blood until it becomes distinctly cherry-red. When examined with the spectroscope at the proper dilution it gives almost exactly the same absorption-bands as the oxyhæmoglobin, only they are a little nearer the violet end of the spectrum. On the addition of ammonium sulphide or Stokes's solution no reduction takes place, however, and the bands remain unchanged.

To distinguish carbon monoxide hæmoglobin from oxyhæmoglobin or to detect the former in the presence of the latter, a great number of reactions have been given, all of which depend upon the greater stability of the carbon monoxide hæmoglobin (the detection of carbon monoxide hæmoglobin in mixtures spectroscopically is difficult and only possible to a certain extent).

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(*a*) **Test of Katayama.** Add 5 drops of blood containing carbon monoxide to 10 cc. of water, then add 5 drops of orange-colored ammonium sulphide and, after mixing, 10 drops of acetic acid or as much as may be necessary to make the mixture faintly acid. With the blood containing carbon monoxide a rose-red color appears, with the normal blood a dirty greenish-gray color. The color is still perceptible with one part of the carbon monoxide blood to five of normal blood.

(*b*) **Test of Kunkel.** Mix carbon monoxide blood with four times its volume of water. To a measured quantity of this mixture add an equal volume of a 3 per cent. tannin solution. Repeat with normal blood and note any difference, especially on standing.

(*c*) **Rubner's Test.** Add to the undiluted blood four to five times the volume of basic lead acetate solution and shake thoroughly for a minute. The carbon monoxide blood turns a beautiful red, the normal blood a brown. After standing for some time the normal blood gradually becomes a chocolate and brownish gray, while the carbon monoxide blood remains unchanged.

The tests (*b*) and (*c*) are especially distinct when the mixtures are allowed to stand for some time in the test-tubes. They show carbon monoxide blood in the presence of normal blood, in the proportion of 1 to 6 or even 1 to 10. They are therefore especially suited to detect small quantities of carbon monoxide in blood.

VIII. Alkaline Hæmatin Solution.

By heating as well as by the action of alkalies or acids the hæmoglobin is split up into coagulated albumin and a pigment. The pigment is different, according as hæmoglobin is decomposed in the absence of oxygen or oxyhæmoglobin in the presence of oxygen (air). In the first case reduced hæmatin (hæmochromogen of Hoppe-Seyler) is formed, in the latter hæmatin or this together with hæmochromogen. This

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cleavage takes place not only in solutions of hæmoglobin, but also in blood itself.

To show the formation of hæmatin add about 1 cc. of caustic soda solution to 8 or 10 cc. of diluted blood (1 : 5), and heat: the solution, which at first is almost cherry-red, turns brown-green. When examined with the spectroscope the light is found to be entirely absorbed up to a part of the red. The spectrum, on further dilution, is not very characteristic; at the proper concentration it consists of a broad, poorly defined band in the orange between C and D. On the addition of one to two drops of ammonium sulphide or Stokes's solution this absorption-band disappears and the two sharply defined and intense absorption-bands of the reduced hæmatin or hæmochromogen (No. 5 in the Table of Absorption Spectra) appear. These have approximately the same position as those of the oxyhæmoglobin, but lie nearer to the violet end of the spectrum. The band nearer to the red is narrower and more sharply defined, that towards the violet is broader, less intense, and not so sharply defined.

IX. Hæmin [1] (Hæmatin Hydrochloride), $C_{32}H_{31}ClN_4FeO_3$.

Small quantities of hæmin are most readily obtained in the following manner: 75 cc. of glacial acetic acid, previously saturated with salt, are heated in a flask on the water-bath to 90°, then 25 cc. of blood are added quite gradually and with constant shaking; continue the heating at 90° for about ten minutes, then pour into a beaker and let stand for twenty-four hours. The hæmin will be found on the bottom of the beaker in the form of an intensely bluish-black layer of glittering crystals. (Examine under the microscope.)

The supernatant fluid is siphoned off, the crystals are washed once with glacial acetic acid, then with acetic acid

[1] Nencki calls this substance acethæmin and gives it the formula $C_{34}H_{33}N_4FeClO_4$.—O.

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and water, and filtered. From the hæmin the hæmatin may be obtained by dissolving it in dilute caustic soda solution, precipitating with dilute hydrochloric acid, filtering, and washing. Since the quantity of the hæmatin thus obtained is very small and sticks to the filter, it is advisable to dissolve it by pouring on the filter a solution of ammonia, and then to

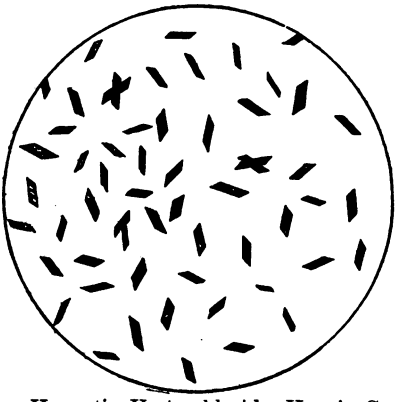


FIG. 4.—Hæmatin Hydrochloride, Hæmin Crystals.

evaporate this solution to dryness on the water-bath. On incinerating, the hæmatin leaves the red oxide of iron (13.5 per cent.).

X. Hæmin Test.

The formation of hæmatin hydrochloride is a very excellent means of recognizing blood-stains. Some blood, dried in the air but not heated, is ground in a mortar with a trace of salt, then boiled in a dry test-tube with glacial acetic acid and the fluid obtained evaporated in a watch-glass on a water-bath heated not quite to boiling. The test may also be made on a microscope-slide. Crush some of the dry blood with a knife-blade, mix it with some salt, put the cover-glass on, let some glacial acetic acid flow under this, and heat the slide over a very small luminous flame till the liquid just begins to boil, then let some more glacial acetic acid flow on

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from the rim, heat again and, after cooling, examine the slide under the microscope. In case no hæmin crystals are found, examine again after allowing to stand for a longer time. We may also let a drop of blood dry on a piece of muslin, then cut out the spot and boil this with glacial acetic acid, etc.

XI. Hæmatoporphyrin.

Hæmatoporphyrin, according to Nencki and Sieber, $C_{16}H_{18}N_2O_3$,[1] is formed from hæmatin by warming the solution of hæmin in glacial acetic acid saturated with hydrobromic acid gas, according to the equation [1]

$$C_{32}H_{32}N_4FeO_4 + 2HBr + 2H_2O = 2C_{16}H_{18}N_2O_3 + FeBr_2 + H_2.$$

The anhydride, $C_{32}H_{34}N_4O_5$, is formed by the action of concentrated sulphuric acid upon hæmatin or hæmoglobin. Add to 8 or 10 cc. of concentrated sulphuric acid, drop by drop, and with constant shaking, five drops of blood. The clear red-violet solution which results shows when examined with the spectroscope two very beautiful and characteristic absorption-bands: a narrow band in the orange and a broader one in the yellow and green (No. 6 in the Table of Absorption Spectra). These show some resemblance to the bands of oxyhæmoglobin, but lie nearer to the red end of the spectrum. The broad band is especially characteristic in that it consists of two parts, a less intense part towards the narrow band and a deep-black part on the other side; frequently the less intense part of the broad band shows an edge towards the red, which is marked by stronger absorption, so that we may also speak of three absorption-bands in the spectrum.

[1] According to Zaleski the formula for hæmatoporphyrin hydrochloride is $C_{34}H_{38}O_6N_4 \cdot 2HCl$, and the formation of hæmatoporphyrin from hæmin is represented by the following equation:

$$C_{34}H_{33}O_4N_4FeCl + 2HBr + 2H_2O = C_{34}H_{38}O_6N_4 + FeBr_2 + HCl.$$

Zeitschr. f. physiol. Chem. **37**, 74.—O.

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XII. Coagulation of the Blood by Heating.

Diluted blood heated to boiling and filtered.

Colorless filtrate, tested for sugar and salts.	**Coagulum, colored brown from hæmatin.**

In every investigation of the soluble constituents of the blood it is necessary first to precipitate the proteids. This is accomplished either by pouring the blood into four times its volume of absolute alcohol or by heating to boiling.

Heat a mixture of 30 to 50 cc. of blood and six to eight times its volume of water over a free flame, and with constant stirring, to vigorous boiling, taking care to keep the reaction neutral or very faintly acid by the cautious addition of dilute acetic or sulphuric acids, then filter. The filtrate should be clear and colorless, which is only possible with fresh blood. The solution is evaporated to a small volume, then divided into halves, one of which is used to perform Trommer's test with some freshly mixed Fehling's solution, the other is further evaporated on the water-bath, a drop allowed to evaporate on a microscope-slide and examined under the microscope: crystals of sodium chloride. Evaporate the residue to dryness and incinerate: salts, especially sodium chloride. Test for chlorides with silver nitrate, for phosphates with ammonium molybdate. The test for sugar frequently does not succeed with commercial blood.

The brown-colored coagulum obtained is washed, well pressed, ground in a mortar with 100 cc. of absolute alcohol, 3 to 5 cc. of concentrated sulphuric acid gradually added, ground again, then the mixture is placed in a flask and heated on the water-bath. A brown-colored solution results and an almost colorless residue of coagulated albumin. Filter, and examine the filtrate spectroscopically (No. 4 in the Table of Absorption Spectra). Especially characteristic is the band

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in the red close to the line C. Heat the solution, after adding some tin and hydrochloric acid, in a flask on the water-bath. The solution turns yellowish-red and when examined spectroscopically shows a dark band, usually not sharply defined, between the green and the blue, similar to that of urobilin. Sometimes, however, only a diffuse darkening in that part of the spectrum is apparent.

The same pigment appears to be present sometimes in urine; at least many urines show similar absorption phenomena.

(*b*) Blood-fibrin.

I. Conduct towards 0.27 per cent. Hydrochloric Acid.

Pour on a few shreds of fresh fibrin (or fibrin kept in glycerin, after it has been well washed) some 0.27 per cent. hydrochloric acid (3 cc. of hydrochloric acid, sp. gr. 1.19, to 500 cc. of water). The fibrin swells gradually and dissolves on long digestion at 40° with the formation of acid albumin. This precipitates on neutralizing with sodium carbonate solution.

II. Conduct towards Hydrogen Peroxide.

Pour on a few shreds of fresh fibrin some hydrogen peroxide solution: evolution of oxygen. Repeat the experiment with boiled fibrin: no gas is evolved. The action of the fibrin on the hydrogen peroxide is probably due to the presence of leucocytes in the fibrin.

III. Conduct towards Salts.

Fresh fibrin swells and gradually dissolves more or less completely in a solution of potassium nitrate.

(*c*) Blood-serum.

The blood-serum, as well as the serous fluids, contains a proteid soluble in water, serum albumin, and one insoluble

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in water, serum globulin, kept in solution by the salts and the alkali of the serum.

I. Precipitation of the Proteids by Salts.

Grind in a mortar 20 cc. of blood-serum with an excess of ammonium sulphate (about 15 g.) for a considerable time, or repeat the process, so that the fluid is completely saturated with ammonium sulphate: by this means both the proteids are precipitated. Filter through a dry filter. The filtrate is perfectly free from albumin; when heated to boiling and acidified with acetic acid it remains clear.

II. Separation of Serum Albumin and Serum Globulin.

Fifty to one hundred cubic centimeters of blood-serum (or serous transudate) are treated with an equal volume of a saturated solution of ammonium sulphate, filtered and washed with a half-saturated solution of the salt. In the filtrate the serum albumin will be found (shown by heating to boiling); the precipitate is serum globulin. It dissolves when brought into water owing to the ammonium sulphate adhering to it; this solution coagulates on heating. For the preparation of pure serum albumin or serum globulin this method is not suitable, since the adhering salts can only be separated by dialysis, and the removal of ammonium sulphate by this means is only accomplished with great difficulty. Therefore it is necessary to saturate the blood with pulverized magnesium sulphate and wash the precipitate with a saturated solution of the same salt.

Both methods are generally regarded as being of equal value. They are, however, not quite equivalent: the quantity of precipitate obtained by the use of ammonium sulphate is larger than that with magnesium sulphate.

The precipitate obtained by means of magnesium sulphate is also soluble in water on account of the magnesium

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sulphate adhering to it. If we subject the solution to dialysis, part of the globulin[1] separates. This is washed with water.

Suspended in water it dissolves on the addition of a trace of caustic soda solution and separates again when the solution is exactly neutralized with dilute hydrochloric acid. If the quantity of caustic soda used to dissolve the globulin was too great, then the globulin is not reprecipitated on neutralization, as it is kept in solution by the sodium chloride formed.

III. Reactions Common to Serum Albumin and Serum Globulin.

For all these reactions use blood-serum which has been diluted with four times its volume of water (20 cc. of blood-serum diluted to 100 cc.[2]).

1. A small portion of the solution when heated to boiling changes but little; it becomes somewhat opaque and white by reflected light, but remains transparent by transmitted light. Coagulation does not take place until the fluid is neutralized by cautiously adding dilute acetic acid; a slight excess of acetic acid redissolves the precipitate. The solution becomes quite clear on warming. On the addition of a few drops of concentrated salt solution a flocculent precipitate of albumin is formed.

2. To a little of the solution in a test-tube add about one-half the volume of a concentrated solution of salt and divide into two approximately equal parts. One-half is heated to boiling: coagulation takes place. To the other half add acetic acid to distinctly acid reaction: it becomes cloudy even

[1] The quantity of the insoluble globulin which separates is always very small; according to recent investigations (Freund, Marcus: Zeitschr. f. physiol. Chem. 28, 559), this is due to the fact that globulin is for the most part soluble in water.

[2] Correspondingly diluted pathological transudates (ascitic or dropsical fluids) may also be used.

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in the cold, and on heating gives a flocculent precipitate. The greater the amount of salt in the albumin solution the less is the coagulation on heating dependent upon the reaction of the fluid; the smaller the amount of salt the nearer must the reaction be to neutral or faintly acid in order that coagulation should take place on heating.

3. Add nitric acid to a small portion of the solution: a precipitate forms at first, which disappears on shaking, then on the addition of more nitric acid a permanent precipitate is formed, which does not dissolve on heating, but turns yellow owing to the formation of the so-called xanthoprotein.

4. If we add glacial acetic acid to a small portion of the solution and heat, acid albumin (acid albuminate) results. Cool and add caustic soda solution. While the reaction is still acid a precipitate of acid albumin forms, soluble in an excess of caustic soda.

5. Heat a little of the solution with half its volume of caustic soda solution. Alkali albuminate is formed. Cool and neutralize with dilute sulphuric or acetic acid. Albuminate is precipitated, partially soluble on heating with an excess of the reagent.

6. If we add copper sulphate solution to some of the dilute blood-serum, a bluish-white precipitate of copper albuminate results, which dissolves on the addition of caustic soda solution to a deep-blue fluid. (The salts of the other heavy metals also as a rule give precipitates.)

7. Add some mercuric chloride solution: heavy white precipitate insoluble in an excess of the precipitating reagent, but soluble in concentrated sodium chloride solution.

8. If we add to some of the solution a few drops of nitric acid till a permanent precipitate is formed and then add absolute alcohol until the volume of the mixture has been doubled, the greater part of the precipitate dissolves (distinction from egg-albumin).

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9. When concentrated nitric acid of 1.48 specific gravity is added to some of the solution, the precipitate first formed redissolves to a clear bright-yellow fluid as soon as the volume of nitric acid added is equal to half the volume of the albumin solution (distinction from egg-albumin).

10. On shaking some of the solution with an equal volume of ether, no coagulation, or at least only a very slight one, results (distinction from egg-albumin).

11. Add to some of the solution half its volume of caustic soda of 1.34 specific gravity and a few (3) drops of a neutral solution of lead acetate and heat: it turns brown or black (distinction from egg-albumin, which gives a much darker solution); when acidified with hydrochloric acid there is soon obtained a grayish-yellow cloudy fluid (distinction from egg-albumin). The reaction depends on the splitting off of sulphur and the formation of lead sulphide.

Dilute the albumin solution with nine times its volume of water (10 cc. diluted to 100 cc.) for the following experiments:

Reactions of very Dilute Solutions of Albumin.

1. Heat to boiling: no change. Then add nitric acid and heat again: precipitation of coagulated albumin.

2. Add acetic acid and potassium ferrocyanide solution: cloudiness and then a flocculent precipitate.

3. Acidify with hydrochloric acid and then add phosphotungstic acid: voluminous gelatinous precipitate.

4. With tannin solution, precipitation takes place, and also,

5. With mercuric chloride solution (soluble in sodium chloride solution).

6. Add some Millon's reagent and heat to boiling: coagulum, which gradually turns reddish to brick-red. This reac-

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tion depends upon the presence of the tyrosine group in albumin. It is also given by all benzene derivatives, which contain a hydroxyl group in place of a hydrogen atom of the benzene nucleus.

For the reactions of coagulated albumin see chapter on Milk, page 4.

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CHAPTER V.

PATHOLOGICAL TRANSUDATES, CYSTIC FLUIDS.

I. Examination for Coagulable Albumins.
II. Examination for Proteids Precipitated by Acetic Acid and Insoluble in Excess of the Reagent.
III. Examination for Albumin and Globulin.
IV. Examination for Urea.
V. Examination for Sugar.
VI. Examination for Paralbumin.

I. Examination for Coagulable Albumins.

See in this connection the chapter on Blood, page 48. It is to be noted that in case of strongly alkaline transudates containing but little albumin no coagulation may result on heating. In such cases the addition of the acetic acid after the heating must be done exceedingly carefully, and in case no precipitate appears it is advisable to add 1 to 2 cc. of concentrated salt solution.

II. Proteids Precipitated by Acetic Acid.[1]

To about 100 cc. of the clear fluid add acetic acid until the reaction is distinctly acid; if a precipitate insoluble in excess of the reagent is formed, the fluid contains mucin or nucleoalbumin. Filter off the precipitate, wash, then grind the

[1] In case pathological fluids are not available, prepare an extract from the thymus gland of the calf (1 part of the finely divided gland treated with ten parts of cold water for 24 hours and shaken from time to time and then filtered), and mix this with an equal volume of blood-serum or ascitic (dropsical) fluid.

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moist precipitate in a mortar with water, adding some sodium carbonate solution; if it does not dissolve, add a small quantity of caustic soda solution. Filter, precipitate again with acetic acid, and wash the precipitate with water.

To distinguish mucin from nucleoalbumin we may use the conduct of the precipitate on heating with hydrochloric acid (see 1 below); mucin forms under these conditions a substance which reduces copper oxide in alkaline solution, while nucleoalbumin does not; nucleoalbumin contains phosphorus, while mucin does not (see 2 below). To test for phosphorus fuse the substance with soda and saltpeter, whereby the phosphorus forms alkali phosphate. The presence of phosphoric acid in the fused mass is only to be regarded as proving the presence of nucleoalbumin, when the precipitated proteid is free from phosphates, especially calcium and magnesium phosphates, and from the very widely distributed lecithin, which contains phosphorus.

The object of dissolving the precipitate in alkali solution, filtering, and reprecipitating with acid is to remove the phosphates as completely as possible.

1. Shake half of the moist precipitate of proteid with a mixture (about 25 cc. or somewhat more) of three volumes of water and one volume of hydrochloric acid, and heat in a flask on the wire gauze to boiling, or heat part of the mixture in a test-tube. Boil gently for about ten minutes, let cool, make a portion alkaline with caustic soda solution without filtering, then test with Fehling's solution, or add a small quantity of copper sulphate solution, shake thoroughly, heat to boiling, and cool the tube by placing it in water: in the presence of mucin red cuprous oxide will be precipitated.

2. Grind the other half of the precipitate in a mortar with absolute alcohol, put the mixture into a flask, heat on the water-bath to boiling, filter, and wash with some alcohol. Press the moist precipitate between filter-paper, put it into

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a dry flask and pour ether over it (or, better, grind it in a mortar with ether and then put the mixture into a flask), shake vigorously, let stand for some time, then filter, and wash with ether. Then grind 0.3 to 0.5 g. of the dry precipitate with thirty times its weight of a mixture of three parts of potassium nitrate and one part of sodium carbonate and fuse the mixture. (See in this connection the test for phosphorus in casein in the chapter on Milk, page 9.)

The presence of phosphoric acid in the fused mass shows that we have to do with nucleoalbumin. It is advisable to make a part of the nitric acid solution of the fused mass alkaline with ammonia: no cloudiness due to calcium phosphate and no crystalline precipitate of ammonium magnesium phosphate should appear. Since, however, the complete removal of calcium phosphate is only accomplished with great difficulty, no attention should be paid to the presence of a trace of phosphoric acid in the fused mass. This may come from the calcium phosphate, or also from traces of adhering lecithin. If we wish to be quite sure of the absence of lecithin, it is recommended to treat the product once more with hot absolute alcohol before fusing it with the oxidizing mixture, evaporate the alcoholic extract to dryness, and fuse the residue with soda and saltpeter. In this fused mass there must be no phosphoric acid.[1]

III. Examination for Serum Albumin and Globulin.

This is done according to the method given under Blood-serum, page 58.

IV. Examination for Urea.

Exactly neutralize 100 cc. of the fluid with acetic acid, then pour into 400 cc. of 95 per cent. or absolute alcohol,

[1] In regard to the detection of the xanthine bases and the pentose group, which is present in very many nucleoalbumins, see the chapter on Pancreas, page 76.

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shake or stir thoroughly, and after twenty-four hours filter. Wash the coagulum with alcohol, evaporate the filtrate to dryness at a low temperature on the water-bath, dissolve the residue in absolute alcohol, filter, evaporate to dryness, and again dissolve the residue in absolute alcohol. If it now dissolves perfectly clear, evaporate the alcoholic solution to dryness again; if it does not, the treatment with absolute alcohol is repeated. The residue obtained by the evaporation of the alcohol is treated, after cooling, with a few drops of nitric acid, and allowed to stand for twenty-four hours in the cold. Usually on the addition of the nitric acid a cloudiness is first formed, caused by the fatty acids, which come from the soaps almost always present; gradually urea nitrate crystallizes out.

If we wish to get rid of these fatty acids, which contaminate the urea nitrate, we put in the treatment with basic lead acetate. The fluid obtained by concentrating the first alcoholic extract, which is generally turbid, is treated with a solution of basic lead acetate, drop by drop, as long as the precipitate continues perceptibly to increase, and then a little ammonium carbonate solution is cautiously added. The fluid above the flocculent precipitate now becomes quite clear. Filter and pass a rapid stream of hydrogen sulphide into the filtrate, filter again after the lead sulphide has well settled, evaporate the filtrate to dryness, and dissolve the residue again in a small quantity of absolute alcohol, etc.

Examine under the microscope the urea nitrate which separates (compare with Fig. 7 in the chapter on Urine, page 95). Then after completely drying the crystals by means of filter-paper or on a porous-clay plate and washing them with some ether, dry, and heat a small quantity on platinum-foil or on a crucible-cover: violent decomposition or explosion. In case the amount of the urea nitrate suffices, convert the remainder into urea (see chapter on Urine, page 95), and test this by means of its reactions.

The amount of urea contained in pathological transudates

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and exudates is very small; if it is present in considerable quantities it indicates a direct connection of the fluids with the kidneys or urinary ducts.

If the test for urea in this way fails, which may happen when the fluid is not quite fresh, in order to practice the method add to 100 cc. of the fluid 0.1 to 0.2 g. of urea (previously dissolved in water).

Under some circumstances, especially when the organs are under examination (in retention of the constituents of urine), the urea nitrate may be mixed with hypoxanthine nitrate: this admixture may be readily detected by dissolving a weighed quantity of the urea nitrate in water and adding ammonia and silver nitrate. Filter off the precipitate, wash, incinerate, and weigh. We thus obtain the weight of the silver corresponding to the amount of hypoxanthine. From this the amount of hypoxanthine nitrate may be calculated, and this is then subtracted from the weight of urea nitrate taken.

V. Examination for Sugar.

Proceed at first just as in the examination for urea, or coagulate 50 to 100 cc. after the addition of a few drops of acetic acid (in case the fluid contains considerable albumin it is to be diluted with one or more times its volume of water), filter, and concentrate, taking care that the reaction does not become alkaline, by adding a few drops of acetic acid when necessary. With the solution obtained, amounting to about 15 cc., after filtering once more, try the α-naphthol test, the Trommer's test, and also the fermentation test (see in this connection the chapter on Urine, "Detection of Sugar," page 118). To obtain a positive result with the fermentation test usually more than 100 cc. of the fluid are necessary.

VI. Detection of Pseudomucin (Paralbumin) in Cystic Fluids.

1. Add to a small quantity of the fluid (about 25 cc.) some drops of an alcoholic solution of rosolic acid, heat to boiling,

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and drop in very dilute sulphuric acid (tenth-normal sulphuric acid) until the color changes to yellow, showing that the fluid has a faintly acid reaction. Heat again to boiling and filter: in the presence of paralbumin the filtrate will be cloudy.

2. Precipitate the same volume (25 cc.) of the cystic fluid with three times its volume of 95 per cent. alcohol, filter, wash a few times with alcohol, press the precipitate between filter-paper, then shake it thoroughly with a mixture of one volume of hydrochloric acid and three volumes of water, and proceed as with mucin (see page 71). In the presence of pseudomucin we obtain a precipitate of red cuprous oxide. Pseudomucin is not precipitated by acetic acid and is thus distinguished from mucin.

A complication may arise if glycogen is also present. This may be detected by treating a part of the precipitate, produced by alcohol, with water and saliva and testing for sugar. If glycogen is present, then the entire quantity of the precipitate is to be treated with saliva and the precipitation with alcohol repeated (Hammarsten).

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CHAPTER VI.

SALIVA AND SALIVARY DIGESTION.

I. Conduct of Saliva towards Reagents.
II. Detection of Mucin.
III. Detection of Potassium Sulphocyanate.
IV. Detection of Ptyalin.
V. Isolation of the Products of Salivary Digestion.

I. CONDUCT OF SALIVA TOWARDS REAGENTS.[1]

1. The addition of acetic acid causes a precipitate insoluble in an excess of the acid due to the presence of mucin.

2. Addition of nitric acid: flocculent precipitate, on heating yellow color; on the addition of an excess of caustic soda solution the yellow color becomes more intense or changes to orange. The reaction is due to mucin (and albumin?).

3. Shake a little saliva with an equal volume of water and some Millon's reagent: white precipitate, which gradually turns red on boiling (mucin).

4. Add caustic soda solution and then a very small quantity of a dilute copper sulphate solution: violet color in consequence of the presence of mucin.

II. DETECTION OF MUCIN.

Twenty cubic centimeters of saliva are poured into 100 cc. of absolute alcohol and thoroughly stirred. The white flocculent precipitate is filtered off, washed with alcohol, then

[1] Use small quantities for the tests.

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once with ether, and the filter placed in a desiccator for twenty-four hours.

1. A small portion of the chalky-white substance is treated with water: it swells up and becomes glassy without dissolving; on the addition of a drop of caustic soda solution it gradually dissolves. This solution gives the biuret reaction with caustic soda and a little copper sulphate solution.

2. Boil for a few minutes, in a test-tube with dilute hydrochloric acid (one part hydrochloric acid to two to three parts of water), the greater part of the substance obtained, cool, then make alkaline with caustic soda, add a little copper sulphate solution and heat to boiling: precipitation of cuprous oxide, which is more noticeable when the tube is cooled in water. The reducing substance split off by boiling the mucin with hydrochloric acid is not sugar [1] (mucose of Fr. Müller).

III. Detection of Potassium Sulphocyanate.

Add one drop of hydrochloric acid to a small quantity of the saliva, then a few drops of a very dilute solution of ferric chloride, and shake thoroughly: red coloration in consequence of the formation of soluble ferric sulphocyanate, $Fe(SCN)_3$.

IV. Detection of Ptyalin.

One gram of starch is made into a paste with 100 cc. of water (for details of the method see the chapter on Pancreas, page 76).

1. After it has cooled to about 40° add to about 10 cc. of the paste approximately 1 cc. of saliva, and shake thoroughly. The mixture becomes clearer and thinner after a few minutes. To a part of the solution obtained add a drop of iodine solution (iodine dissolved in an aqueous solution of potassium iodide): no blue coloration, but either a red color (due to erythrodextrin) or merely a yellow color from the iodine.

[1] It is chitosamine, see Zeitschr. f. Biolog. 42, 468 (1901).—O.

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With the other half of the solution try the Trommer's sugar test. As a check repeat the same experiment with boiled saliva: the fluid does not become clear, the starch remains unchanged.

2. Repeat experiment 1, but digest for an hour at 40°, then shake the solution obtained with some yeast, fill a fermentation-tube with it, and let stand at about 35°: the sugar, maltose, formed in the salivary digestion is fermentable.[1]

Influence of Acids on the Diastatic Action of Ptyalin.

(*a*) Shake together thoroughly 10 cc. of starch paste and 1 cc. of 0.27 per cent. hydrochloric acid (6 cc. of hydrochloric acid, 1.19 sp. gr., diluted to one liter), add 1 cc. of saliva, and digest on the water-bath at 40°. The diastatic action of the enzyme is completely prevented by the acid.

(*b*) Repeat the experiment with 1 cc. of dilute acetic acid of 0.5 to 1 per cent. (1 to 2 cc. of glacial acetic acid to 200 cc. of water): the diastatic action is not prevented, but it is perceptibly retarded. An excellent arrangement of these experiments is also the following:[2]

The mixtures of starch paste and saliva and in the given case also the acid are placed in test-tubes in a water-bath whose temperature is kept at 40° to 42°. Each test-tube has a pipette standing in it. From time to time a drop of the mixture is taken out and brought into contact with a dilute solution of iodine in a solution of potassium iodide. For this purpose place beforehand a number of drops of the iodine solution at regular intervals (in rows) on a porcelain plate. If we proceed in this manner, the addition of a drop of the mixture to the iodine solution requires only the smallest amount of time, so that the time interval which elapses

[1] The yeast contains maltase, which inverts the maltose. The glucose formed then ferments with the yeast.—O.

[2] Virchow's Arch. 120, 343.

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between the taking out of drops from each of the three mixtures may be disregarded, if we work quickly.

In order to obtain a better and clearer insight into the progress of the process, select for each new test of the mixtures a new row of drops of the iodine solution on the plate. This arrangement enables us even to recognize erythrodextrin, if it is present in sufficient quantity, alongside of the starch. In this case besides the blue color due to starch the red color due to erythrodextrin will appear when the drops begin to dry. Test the portions taken out later also for sugar; a very small quantity is sufficient for this.

V. Isolation of the Products of Salivary Digestion.

Make 25 g. of starch into paste with one liter of water, stirring constantly. After cooling to 40° (in order to determine the temperature, the thick paste must be well stirred, as the distribution of the temperature in it is very unequal), add 25 cc. of saliva, and stir thoroughly (it is advisable to use saliva that has been collected the previous day, as its activity appears to be greater). The paste very soon liquefies. As soon as this has happened pour the paste into a cylinder and digest for two and a half to three hours at 40°. After this time, as a rule, the starch will have disappeared, the solution no longer gives the reaction for starch with iodine. An absolutely exact statement of the time necessary cannot be made, as the activity of the saliva is not always the same. If the starch has not entirely disappeared, the digestion must be continued. It is not advisable to carry the digestion to an end in the dish originally used, since the formation of solid masses of the paste on the side of the vessel is scarcely to be avoided, and these resist the digestive action of the saliva for a long time. This again might lead to a false impression concerning the progress of the digestion. After the digestion is completed heat the solution on the water-bath, filter from

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a small quantity of material resembling cellulose, which comes from the starch, evaporate on the water-bath to about 100 cc., filter again, and evaporate to about 25 cc Pour this sirup while still hot into a flask containing 100 cc. of 90 per cent. alcohol heated to boiling on a water-bath, heat a little longer, shake thoroughly, and let stand till next day. The precipitate consists of dextrin with some maltose; the alcoholic solution contains principally maltose with a little dextrin and traces of glucose. A perfect separation is not attainable by a single precipitation with alcohol, and also requires a larger amount of material. On the next day pour off the alcoholic solution and wash once with alcohol the glutinous precipitate sticking to the flask.

(*a*) Pour on the residue in the flask about 50 cc. of water, heat to boiling (with constant shaking so that the precipitate may not be burned or heated too highly), and boil the solution until the odor of alcohol has completely disappeared. Let cool, dilute to 100 cc., and filter through a dry filter.

A small portion of this solution turns blue-violet on the addition of a very small quantity of iodine solution, and red with a larger quantity. Sometimes these colors with iodine are not given because achroödextrin has been formed. The solution gives the reactions for sugar very markedly.

Thirty cubic centimeters of the solution obtained are diluted to 150 cc. Add 10 cc. of hydrochloric acid to 100 cc. of the solution and mark this solution A. The remainder, 50 cc., is used to determine the rotation. It amounts to, say, 6.8 per cent. calculated as glucose.

The solution A is then heated to boiling and kept boiling gently for twenty minutes. Let cool, fill up to the former volume (100 cc.), and again determine the rotation (neutralization of the solution may be omitted, if we determine the rotation rapidly and clean out the observation-tube at once;

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if we wish to neutralize, this must of course be done before the volume is diluted to 100 cc.). The rotation will be found to have very materially decreased; it amounts to only about one-third of the former rotation; in the case given to about 2.6 per cent. The decrease in the rotation is due to the transformation by boiling with acids of the very strongly dextrorotatory dextrin (and maltose) into the less strongly rotating glucose.

(*b*) The alcoholic solution, when evaporated on the water-bath, yields a very sweet-tasting sirup, which gradually dries up, containing maltose with a little dextrin and a very small quantity of glucose. The maltose crystallizes out of this sirup only with great difficulty. It crystallizes more readily if the digestion of the starch paste with the saliva be continued for a longer time (twenty-four hours). On heating with acids the maltose, like the dextrin, yields glucose. This conversion may be shown as in the case of dextrin by the decrease in rotation. For this purpose dissolve 3 g. of the residue in hot water, let cool, dilute to 150 cc., determine the rotation of the solution, then treat 100 cc. of this solution just as in the case of dextrin, and determine the rotation once more. It amounts to not quite half as much as before boiling with the acid.

Maltose, $C_{12}H_{22}O_{11}+H_2O$, is formed together with dextrin by the action of malt (germinated barley) on starch by virtue of a ferment (diastase) contained in the malt. Maltose crystallizes in fine needles, it is strongly dextrorotatory (specific rotation 139.2°), reduces Fehling's solution, is fermentable,[1] and is found in beer. To show the perfect fermentability, add to about 50 cc. of a 2 per cent. solution some yeast, let stand twenty-four hours at about 35°, filter, and test the filtrate for sugar by means of Trommer's test: no precipitate of cuprous oxide is formed.

[1] According to E. Fischer, maltose is first converted into glucose by the maltase of the yeast and this then ferments.—O.

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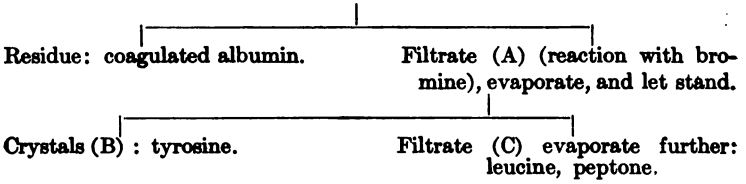
CHAPTER VII.

EXAMINATION OF THE PANCREAS.

I. Tryptic Digestion.
II. Diastatic Action.
III. Cleavage of Fats.
IV. The Nucleoproteid of the Pancreas.

I. TRYPTIC DIGESTION.

Digest 250 g. of fibrin with 1 liter of alkaline chloroform-water and 2 to 2.5 g. of pancreas-powder for 48 hours, add acetic acid, boil, and filter.



The best material is fresh fibrin. If only the coagulated material preserved in chloroform-water is available, it is advisable to heat it with acidified water (3 cc. of hydrochloric acid, specific gravity 1.19, to 1 liter of water), and then wash it thoroughly on a muslin filter. Sometimes this treatment is omitted. The digestion takes place with the unswollen fibrin also, but not so well. The chloroform-water is prepared by shaking 1 liter of water with 5 cc. of chloroform. The chloroform is used to prevent putrefaction: thymol may also be used for this purpose. If antiseptic material is not used, very marked putrefaction is certain to result. The pancreas-powder is prepared according to the method of Kühne. Pancreas[1] which has lain for twenty-four hours is carefully freed from all visible fat, ground with absolute alcohol, filtered after standing a short time and pressed,

[1] From the ox or dog.

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ground with ether, again filtered and pressed, dried in the air by allowing the ether to evaporate; again ground and sifted through wire gauze, using only the powder that passes through the sieve. Instead of using the powder directly we may also digest it for a day at 40° with chloroform-water (2.5 g. : 100 cc.), to which some drops of sodium carbonate solution have been added, and use the filtrate for the experiment. The action is usually somewhat weaker; in many cases, however, such a solution is to be preferred. Kühne prepares an extract from the pancreas-powder by digesting for three or four hours one part of the powder with five to ten parts of a salicylic acid solution (0.1 per cent.), and then filtering.

The mixture of fibrin, pancreas-powder, and chloroform-water must have a distinctly alkaline reaction. When fresh fibrin is used the addition of 5 cc. of a concentrated sodium carbonate solution is sufficient. When coagulated fibrin which has been previously treated with dilute hydrochloric acid is used it may easily retain some of the acid, and in this case the 5 cc. of sodium carbonate is insufficient. We must then repeatedly add sodium carbonate solution, until even after standing for a considerable time the mixture has a distinctly alkaline reaction.

The mixture is digested at 40° in a glass-stoppered bottle for forty-eight to seventy-two hours and should be repeatedly shaken thoroughly during the digestion. After the digestion make the contents of the bottle quite faintly acid with acetic acid, heat to boiling in an enamelled-iron dish or tinned vessel, and filter.

1. To a small portion of this filtrate (A) add, drop by drop, and with constant shaking, some bromine-water: the fluid takes on a violet color: tryptophan reaction. The rest of the filtrate is evaporated to a thin sirup (about 100 cc.) and let stand for some days in a cool place. A considerable quantity of white granular crystals of tyrosine separates (B). Decant through muslin, put the tyrosine into a dish or beaker and wash it quickly a few times by decantation, then put it into a flask and heat with water to which a little ammonia has been added, and filter. The hot filtrate is evaporated on the

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water-bath until the ammonia has disappeared; on cooling the tyrosine separates as a chalky mass; this is filtered off, washed, and dried on filter-paper.

Tyrosine, $C_9H_{11}NO_3$, p-oxyphenyl α-amino-propionic acid, $C_6H_4 < {}^{OH\ (1)}_{CH_2CH(NH_2)COOH\ (4)}$, is a constant cleavage product of albumin and horn substance (but not of gelatin or of tissues which may be converted into gelatin), formed by the action of dilute acids or alkalies and also on putrefaction. It forms shining silky needles, melting at 310–314° with decomposition. It is very difficultly soluble in cold water, slightly in hot water, insoluble in alcohol and ether, soluble in ammonia and caustic alkalies.

Reactions of Tyrosine.

(*a*) Heat a small portion in a test-tube with some water to boiling, let cool slowly (without cooling in water), and examine the crystals under the microscope: tufts of needles, usually of very regular form, which readily dissolve on the addition of hydrochloric acid and do not melt when warmed gently on the slide of the microscope (distinction from the needles of fatty acids in old pus, etc., which often resemble tyrosine needles, and from the needles of fat, which form oily drops on warming and do not dissolve in hydrochloric acid).

(*b*) Suspend a small quantity of tyrosine in water in a test-tube, add a few drops of Millon's reagent and heat gently till boiling begins. The mixture at first turns rose-red, then gradually deep red, frequently, however, not till it has stood some time; if the quantity of the tyrosine is somewhat large, the solution becomes cloudy and gradually deposits a red precipitate. Strong boiling is not advisable, as the reaction is then often not so good and the color is more brownish. All benzene derivatives in which a hydrogen atom of the benzene nucleus is replaced by a hydroxyl group give the same reaction.

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(*c*) **Piria's Test,** due to the formation of tyrosine sulphonic acid. Pour on a little tyrosine in a dry test-tube some concentrated sulphuric acid, place the tube in an actively boiling

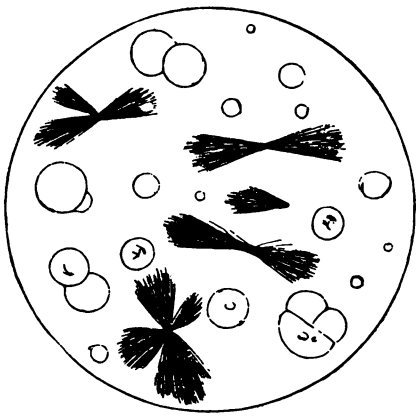


FIG. 5.—Leucine and Tyrosine.

water-bath, and leave it there for about half an hour. Let cool, pour into several times its volume of water, rinse with water, and grind the solution, diluting, if necessary, with barium carbonate, added in portions, until the solution no longer reacts acid. Filter, evaporate to a few cubic centimeters, and add cautiously some very dilute ferric chloride solution: violet color.

(*d*) **Reaction of Denigès.** Gently heat a small portion of the tyrosine with 2 to 3 cc. of concentrated sulphuric acid to which a few drops of formalin have been added: brownish-red color, which turns green on the addition of glacial acetic acid. Neither albumin nor peptone reacts with this reagent of Denigès.[1]

[1] According to C. T. Mörner the reagent is made as follows:

1 volume formalin;
45 volumes distilled water;
55 volumes concentrated sulphuric acid.

If a portion of this solution (2 cc.) is treated with a little tyrosine (in the solid form or in solution) and the mixture is heated to boiling, a beautiful green color appears. Zeitschr. f. physiol. Chem. **37**, 86.—O.

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2. The solution C decanted from the tyrosine is evaporated further on the water-bath: crystals of leucine form on the surface. These are to be examined under the microscope: weakly refracting balls or aggregates, sometimes with a recognizable radiating structure, which dissolve readily in hydrochloric acid and caustic alkalies.

The solution is evaporated to a sirup, treated with several times its volume of 90 per cent. alcohol, put into a flask, heated on the water-bath to boiling, and, after it is perfectly cold, filtered. The alcoholic extract contains a good deal of leucine besides a little peptone; the insoluble residue, much peptone with a small quantity of leucine. The alcoholic extract is evaporated to dryness, the residue dissolved in water, the solution boiled with lead hydroxide, and, after cooling, filtered. The filtrate freed from lead by means of hydrogen sulphide is filtered, evaporated to a small volume, and the leucine, which separates on standing, dried on a clay plate.

Leucine, α-amino-isobutyl-acetic acid, $(CH_3)_2CHCH_2$-$CH(NH_2)COOH$, is a constant cleavage product of albumin, horn substance, gelatin, and tissues yielding gelatin, formed by the action of dilute acids or alkalies and also on putrefaction. It forms in pure condition shining white leaflets which are wet by water only with difficultly. It dissolves in 40 to 46 parts of cold water, more readily in hot, and with difficulty in alcohol, but is very much more readily soluble in these solvents in the impure condition.

Reactions of Leucine.

(*a*) A little of the substance, when heated cautiously in an open tube held slantingly, forms a woolly sublimate of leucine; an odor of amylamine is evolved at the same time, a part of the leucine undergoing decomposition.

(*b*) Put a piece of caustic potash (stick form) about 1 cm.

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long into a test-tube with a little leucine and one to two drops of water. Heat till the alkali melts, when a strong evolution of ammonia takes place. Let cool, dissolve the melted mass in a little water, and acidify with dilute sulphuric acid: odor of valeric acid. Leucine by this treatment takes up oxygen and is decomposed into ammonia, carbon dioxide, and valeric acid.

(*c*) Dissolve some leucine in water, decolorize the solution, if necessary, with some good bone-black, filter, make alkaline with caustic soda solution, and then add one to two drops of copper sulphate solution: the precipitate of copper hydroxide, which first forms, dissolves to a blue solution of leucine copper, which is not reduced on heating.

3. The residue insoluble in alcohol, which has been freed more or less completely from leucine, is treated with absolute alcohol, filtered, and washed with alcohol and ether. Test this for albumose and peptone by dissolving it in a little water and saturating with ammonium sulphate, etc. (See chapter on Gastric Digestion, page 33). Ultimately the peptone itself is to be isolated.

II. Diastatic Action of the Pancreas.

Prepare some starch paste as follows: Measure off 100 cc. of water, grind 1 g. of starch (potato-starch) in a mortar with a part of the water, pour the fluid into a dish, rinse the mortar with the rest of the 100 cc. of water, and heat the mixture to boiling with constant stirring. Then treat 1 g. of pancreas-powder with 50 cc. of water, digest for two hours at 40°, and filter. Mix in a test-tube equal volumes of the starch paste and the pancreas extract, and digest at 40°. The starch paste liquefies and becomes transparent. At this point the solution gives the reaction for sugar and no longer turns blue on the addition of iodine, but either turns red (erythrodextrin) or gives no color at all.

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As a check, repeat the same experiment, but boil the pancreas extract before using it: the saccharifying action does not take place.

Instead of the aqueous pancreas extract we may use a glycerin extract of the pancreas (about ten drops to 10 cc. of starch paste), or the fresh pancreas when that is available. Grind a piece of the pancreas (from the ox or dog) with water to a thin paste, filter through muslin, and mix about equal volumes of starch paste and the gland extract.

III. Detection of the Lipolytic Ferment, Lipase.

This succeeds only with the fresh pancreas. Grind the finely minced pancreas to a thin paste, divide it into two equal parts, boil one part (A) to destroy the ferment, but not the other (B). Then shake a few grams of butter-fat with luke-warm water, add a few drops of rosolic acid solution and then tenth-normal caustic soda solution till the mixture is distinctly red. Now mix equal parts of the fat emulsion with the pancreas-paste, one part with (A), the other with (B), and add a drop or two of chloroform. If these mixtures are not distinctly red, then add cautiously, drop by drop, dilute sodium carbonate solution. Digest the mixture twelve to twenty-four hours at 40°. The solution marked (A) does not change its color; (B) becomes yellow in consequence of the butyric acid set free by the hydrolysis of the butyrin.

In like manner we can test cystic fluids, which are suspected to come from the pancreas, for the saccharifying and lipolytic ferments. To detect the presence of trypsin digest a portion of the fluid, made alkaline, if necessary, for twenty-four hours at 40°. Since albumin is a constant constituent of the cystic fluids, they must now contain peptone and tryptophan in case trypsin is present. Coagulate the albumin (adding water if necessary) by heating the solution faintly acidified with acetic acid, filter, and divide the filtrate into two parts. One part is used for the biuret reaction with caustic soda and copper sulphate solutions, the other for the tryptophan reaction. To detect very small quantities of the digestion products of albumin we may

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also precipitate the filtrate or a part of it with phosphotungstic acid, after first acidifying strongly with hydrochloric acid. Then warm the tube, when the precipitate becomes more dense, packs together, and usually sticks to the glass. Rinse it a few times with water, dissolve in dilute caustic soda solution, shake till the blue color, which forms at first, disappears, then cautiously add the copper sulphate solution.

IV. NUCLEOPROTEID OF THE PANCREAS.

The pancreas, according to Hammarsten, contains a nucleoproteid, which, according to Bang, may be split up into albumin and an acid, guanylic acid. The latter yields on cleavage phosphoric acid, guanine, and a carbohydrate containing five atoms of carbon, a pentose of the composition $C_5H_{10}O_5$. The nucleoproteid (nucleoalbumin) itself also yields the same cleavage products, as Hammarsten had already found before Bang.

Detection: Heat 200 g. of finely chopped pancreas to boiling with one liter of water, keep boiling for ten minutes, filter, and add cautiously to the filtrate, while still warm, about 10 to 15 cc. of 30 per cent. acetic acid, until a fine flocculent precipitate begins to settle. If the precipitate does not settle well it is advisable to heat again. Filter, wash with water, remove the precipitate from the filter, grind it with 50 cc. of absolute alcohol, filter, treat the precipitate with about 50 cc. of ether, filter next day, wash once with ether, and grind. The presence of phosphorus and pentose may be very easily shown in the somewhat impure nucleoproteid thus obtained.

1. To detect the phosphorus fuse a small quantity with the oxidizing mixture and then proceed as directed under Casein, page 9.

2. To detect the pentose the phloroglucin and the orcin tests may be used.

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(*a*) **Phloroglucin Test.** Pour on a very small quantity of the substance a few cubic centimeters of hydrochloric acid, add a little phloroglucin, and heat to boiling: cherry-red color, then turbidity. Let cool somewhat, shake with an equal volume of amyl alcohol, and examine this with the spectroscope: absorption-band between D and E.

(*b*) **Orcin Test.** Instead of phloroglucin take a few orcin crystals and proceed in the same way: reddish-blue color, then precipitation of a blue pigment. The amyl alcohol turns red and after some time emerald-green. Examine with the spectroscope: absorption-band between C and D.

If somewhat larger quantities (not less than 0.2 g.) of the nucleoproteid are available, the presence of the guanine may also be shown. Heat in a flask, cautiously and shaking constantly, with about 25 cc. of a mixture of one volume of hydrochloric acid and three volumes of water. Keep boiling gently about fifteen minutes, neutralize with caustic soda solution, acidify with acetic acid, and let stand till next day: guanine will separate. In the filtrate the phosphoric acid may be detected by means of uranium acetate, the pentose by means of the Trommer's test and the pentose tests given above.

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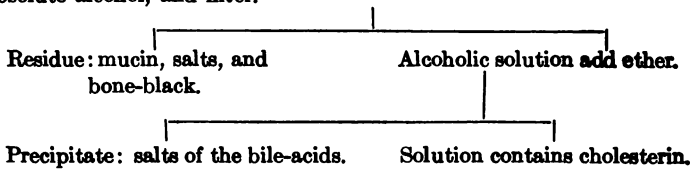
CHAPTER VIII.

EXAMINATION OF BILE.

I. Detection of the Constituents of Bile.
II. Detection of Mucin in Bile.
III. Preparation of Taurine.

I. DETECTION OF THE CONSTITUENTS OF BILE.

Mix 200 cc. of ox-bile with bone-black, evaporate to dryness, heat with absolute alcohol, and filter.



Two hundred cubic centimeters of ox-bile are evaporated as nearly to dryness as possible on the water-bath with some good bone-black (one-fourth the volume), the residue removed from the dish after cooling, put into a flask, and extracted on the water-bath with alcohol. After cooling, filter, evaporate the extract to dryness on the water-bath, dissolve the dry mass in absolute alcohol, and filter into a dry flask. Add to the filtrate anhydrous ether until the cloudiness becomes permanent. After long standing, sometimes even on the next day, a mixture of the sodium salts of glycocholic acid, $C_{26}H_{43}NO_6$, and taurocholic acid, $C_{26}H_{45}NSO_7$, crystallizes (Plattner's crystallized bile).

The bile-acids are distinguished by a reaction which is

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also given by cholic acid (cholalic acid), a cleavage product of both bile-acids.

Pettenkofer's Test for the Bile-acids.

Use either a 1 per cent. solution of the crystallized salts of the bile-acids or of the commercial fel tauri depurat. sicc.

Add to a few cubic centimeters in a test-tube five drops of a 10 per cent. solution of cane-sugar (or add a small piece of cane-sugar and dissolve this by shaking). Then let about half the volume of concentrated sulphuric acid flow slowly down the side of the tube, held in a slanting position, so that the sulphuric acid forms the under layer. At the surface of contact of the two liquids a purple-violet color appears. Dip the test-tube into a cylinder or beaker filled with water and mix the sulphuric acid and the solution of the bile-acids, but not too quickly,[1] by moving the test-tube around the walls of the vessel in circles; a deep purple solution results. For examination with the spectroscope pour a little of the solution into a few cubic centimeters of glacial acetic acid and about the same amount into some alcohol.

(*a*) The acetic acid solution shows an absorption-band in the green and a more or less pronounced greenish fluorescence.

(*b*) The alcoholic solution also shows immediately after mixing only this one band, but very soon it takes on a brownish shade and then shows two pronounced absorption-bands in the green and in the blue.

Modification of the Test according to Mylius.[2]

Instead of the cane-sugar use a drop of furfurol solution (a drop of furfurol shaken thoroughly in a test-tube with 10 c.c. of water). The reaction develops more slowly,

[1] If the temperature exceeds 70° during the mixing the pigment is destroyed.

[2] Zeit. für physiol. Chemie, **11**, 493.

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requires apparently more sulphuric acid, and is often not equal in intensity to the original Pettenkofer's reaction.

According to v. Udránszky[1] the best proportions for this reaction are: 1 cc. of the alcoholic solution of the bile-acids, one drop of the furfurol solution (0.1 per cent.), and 1 cc. of concentrated sulphuric acid.

Modification according to Neukomm.

Use a 0.1 per cent. solution of the salts of the bile-acids. Add to some drops of this solution a trace of sugar solution, then one or more drops of dilute sulphuric acid, and evaporate in a dish on the water-bath: a violet color develops at the edge of the evaporating mixture. As soon as this is distinctly perceptible stop the evaporation.

Preparation of Glycocholic Acid.

Dissolve the rest of the crystallized bile (with the exception of a small part to be kept) in a little water, pour some ether on top of this solution, and then add dilute sulphuric acid until a permanent and marked cloudiness appears. The glycocholic acid separates gradually in fine silky needles. Glycocholic acid is very difficultly soluble in cold water, more readily in hot, and easily soluble in alcohol. The aqueous solution has a bitter-sweet taste, reacts acid, and decomposes the alkaline carbonates when heated with them. The acid as well as the salts are dextrorotatory.

Preparation of Taurocholic Acid.

The preparation of this acid is best accomplished by using dog's bile, in which it is the only acid present. To show its presence in the crystallized bile, fuse 0.2 g. of this

[1] Zeit. für physiol. Chemie, **12**, 371.

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with 6 g. of the oxidizing mixture and test for sulphuric acid in the fused mass (see chapter on Milk, page 7).

Detection of Cholesterin.

Cholesterin is present in the alcoholic-ethereal fluid, together with the salts of the bile-acids still remaining in solution. Allow the greater part of the ether to evaporate by letting the alcoholic-ethereal solution stand in an open dish, remove the rest of the ether and alcohol by evaporation on the water-bath, dissolve the residue in water, shake the mixture with ether, remove the ether and evaporate the ether extract. Try the cholesterin reaction with the residue (see Chapter IX, Examination of Biliary Calculi, page 90).

II. Mucin of the Bile.

On the addition of acetic acid to 100 cc. of bile there is formed a resinous precipitate, which is ordinarily called bile-mucin, but whose exact nature is still doubtful. The precipitate also contains glycocholic acid, which may be removed by extraction with alcohol.

III. Preparation of Taurine.

Heat 300 cc. of bile in an evaporating-dish on the sand-bath with 100 cc. of hydrochloric acid until the resinous mass, which separates at first, the so-called choloidic acid, is converted into dyslysin (anhydride of cholic acid). This point may be determined by drawing out the resinous mass in threads with a glass rod. These should solidify at once and should then be quite brittle. It may then be assumed that all the taurocholic acid is decomposed. Decant from the dyslysin and evaporate until sodium chloride begins to separate, filter, evaporate on the water-bath to a small volume (the salt which separates is to be removed by filtering again), pour the fluid remaining into about fifteen times its volume

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of alcohol (or mix with it), after twenty-four hours filter off the taurine which has separated, wash with alcohol and recrystallize it from hot water, using some bone-black to decolorize. Taurine (amino-ethyl sulphonic acid), CH_2NH_2 | , crystallizes in large, transparent, glittering prisms, CH_2SO_3H which are readily soluble in hot water, more difficultly in cold, and insoluble in absolute alcohol.

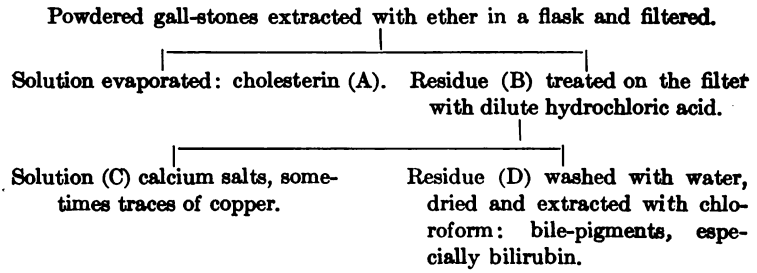
1. Heat a few crystals on platinum-foil: the taurine melts, turns brown and carbonizes on heating more strongly, developing a suffocating odor (sulphurous acid [and sulphuric acid ?]).

2. Powder a few crystals, mix with several times their volume of dry sodium carbonate, and fuse on platinum-foil. After cooling, dissolve the fused mass in water, put it into a test-tube, and add some dilute sulphuric acid: odor of hydrogen sulphide. Moisten a strip of filter-paper with a solution of lead acetate and remove the excess of the solution by pressing between filter-paper. When held over the opening of the test-tube this paper turns brown or black owing to the formation of lead sulphide.

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CHAPTER IX.

EXAMINATION OF BILIARY CALCULI.



Pour on the finely powdered gall-stones [1] (about 2 g.) in a dry flask about ten times the volume of ether, shake a few times, then filter through a dry filter, and evaporate the ethereal solution.

1. The cholesterin thus obtained, $C_{27}H_{45}OH$, is not quite pure, but often contains some fat. It is sufficiently pure, however, for the following reaction: [2]

(*a*) Dissolve a part of the cholesterin in hot alcohol, let the solution evaporate spontaneously on a watch-glass, and examine the mass of cholesterin crystals resulting under the microscope: rhombic tablets, frequently with re-entering

[1] Mixture of cholesterin and pigment-stones.

[2] To purify it, dissolve in 80 per cent. alcohol, add a piece of caustic potash, warm in a flask, on the water-bath, evaporate to dryness in a dish on the water-bath, take up the residue with water, shake the mixture with ether (free from alcohol), remove, and evaporate the ethereal extract.

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angles. These appear beautifully formed when they have separated spontaneously from old exudates, transudates, or cystic fluids.

(*b*) Place a small quantity of the cholesterin crystals on a slide, put on the cover-glass, and let a drop of a mixture of

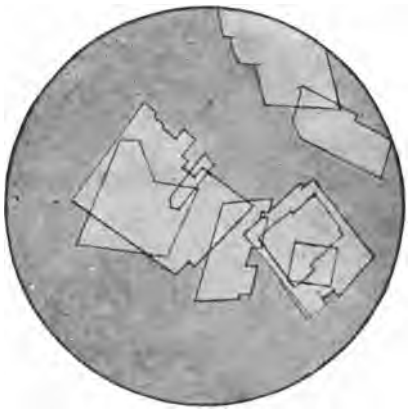


FIG. 6.—Cholesterin from Cystic Fluid.

five volumes of concentrated sulphuric acid and one volume of water flow under the cover-glass from the side and then a very small quantity of a solution of iodine; the cholesterin crystals gradually turn brown or violet, sometimes even a clear blue, and partially dissolve. The coloring never appears entirely uniform and is often incomplete.

(*c*) Use the greater part of the cholesterin for the following reactions:

1. Evaporate a small quantity on the cover of a porcelain crucible with hydrochloric acid and a trace of ferric chloride: blue color.

2. **Chloroform Sulphuric Acid Reaction.** Dissolve some cholesterin in a dry test-tube in a few cubic centimeters of chloroform, add an equal volume of concentrated sulphuric

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acid, and shake thoroughly several times. The chloroform solution turns blood-red, gradually changes to cherry-red, and finally to purple. The sulphuric acid under the chloroform solution shows a greenish fluorescence. If now some of the chloroform solution is poured into a wet test-tube and shaken, it will quickly become colorless, and upon adding sulphuric acid the original red color will be restored. When poured out into a dish the chloroform will also become colorless from the moisture which it abstracts from the air. If the purple-colored chloroform solution is diluted by the addition of more chloroform, it often turns blue (in consequence of a small amount of water in the chloroform), and the addition of sulphuric acid turns it red again. In very dilute solutions (a trace of cholesterin dissolved in chloroform) the reaction is somewhat different, but also characteristic: yellow to rose color of the chloroform, yellow color of the sulphuric acid with a greenish fluorescence.

3. **Liebermann-Burchard Reaction.** Dissolve a small quantity of the cholesterin in a few cubic centimeters of chloroform in a dry test-tube, add two or three drops of acetic anhydride, and then concentrated sulphuric acid, drop by drop. A rose color first develops, then a beautiful blue, which finally turns bluish green. If only a very small quantity of cholesterin is used, the green color develops after standing a few minutes.

Both tests, 2 and 3, are equally delicate. Not only cholesterin but also its esters, such as those of palmitic and stearic acid occurring in nature (Liebreich's lanolin), give the reactions.

If a somewhat larger quantity of cholesterin is available, purify it by repeated recrystallization from hot absolute alcohol, press the crystals between filter-paper, and determine the melting-point. It melts at 145° (distinction from the cholesterin occurring in plants, phytosterin, which gives

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a very similar chloroform sulphuric acid reaction, but whose melting-point is 133–136°).

2. The residue B remaining on the filter is washed a few times with ether, the upper part of the filter, which still contains some cholesterin, is cut off, the filter filled with dilute hydrochloric acid (1 : 3), and the filtrate poured repeatedly upon the residue.

3. The filtered solution C contains calcium salts, which may be proved by making a portion of the solution alkaline with ammonia, and adding acetic acid and ammonium oxalate; sometimes traces of copper are present, as may be shown by the addition of a few drops of potassium ferrocyanide: brown color or precipitate of copper ferrocyanide.

4. The residue D remaining on the filter is washed with water till the wash-water is free from hydrochloric acid, the filter is then dried in an air-bath, cut into pieces, and these are heated in a dry flask with a little chloroform. The brownish-yellow fluid resulting is then filtered through a dry filter.

The solution contains bilirubin, $C_{32}H_{36}N_4O_6$. Let a portion of this solution evaporate spontaneously on a watch-glass and examine the residue under the microscope: small, ill-defined, elongated rhombic plates or indistinct crystalline grains of bilirubin (not to be confused with the cholesterin crystals, which may still be present). Pour on the edge of the watch-glass a drop of nitric acid which contains some nitrous acid: play of colors of the Gmelin reaction (see chapter on Urine, "Bile-pigments," page 121). Bilirubin[1] is isomeric with hæmatoporphyrin (page 56), which gives Gmelin's reaction indistinctly.

Shake up the greater part of the solution with a weak solution of sodium hydroxide: the pigment goes over into the alkaline solution, while the chloroform is more or less

[1] According to Zaleski hæmatoporphyrin has the formula $C_{34}H_{38}N_4O_6$ and is not isomeric with bilirubin, Zeit. physiol. Chem. 37, 74 (1902).—O.

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completely decolorized (distinction from lutein, occurring in the yolk of eggs, corpus luteum, many cysts, blood-serum, palm-oil, and in flowers, which is not removed from the chloroform solution by shaking with dilute alkalies).

Separate the alkaline solution and let it stand in the air: it gradually turns green owing to the formation of biliverdin, $C_{32}H_{36}N_4O_8$(?) (oxidation).

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CHAPTER X.

EXAMINATION OF THE URINE.

I. General Properties.

Note the color of the urine and whether it is clear or cloudy; test the reaction of the fresh urine with litmus paper, and determine the specific gravity with a urinometer. Let a portion stand for some time, note what happens, and again determine the reaction.

II. Conduct towards Reagents.

Treat small portions of urine with the following reagents:

1. **Caustic Soda:** turbidity, precipitation of the phosphates of calcium and magnesium, which, on heating, become more dense and settle to the bottom; they always appear slightly colored; on standing crystals of ammonium magnesium phosphate are also deposited.

2. **Hydrochloric Acid:** dark coloration, especially on warming, sometimes a distinct red coloration; on standing crystalline deposit of uric acid.

3. **On boiling,** the urine remains, as a rule, clear and its reaction acid; frequently, however, it becomes cloudy, due to the precipitation of calcium phosphate. In this case the reaction is either neutral or alkaline. This precipitate readily dissolves on the addition of a few drops of acetic acid, while that due to albumin (which resembles this phosphate precipitate very closely in appearance) remains undissolved.

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4. **Barium Chloride:** white precipitate of barium phosphate and sulphate; on the addition of hydrochloric acid the barium phosphate dissolves and the quantity of the precipitate diminishes perceptibly.

5. **Silver Nitrate:** white precipitate of silver chloride and silver phosphate; on the addition of nitric acid the latter dissolves, silver chloride remains.

6. **Basic Lead Acetate:** heavy precipitate, which consists principally of lead chloride, lead phosphate, and lead sulphate, together with the greater part of the coloring matter of the urine. The filtrate is colorless or almost colorless. The precipitation with basic lead acetate is frequently used to decolorize the urine.

III. PREPARATION OF UREA.

Two hundred to three hundred cubic centimeters of dog's urine or double the quantity of human urine are treated with baryta mixture (one volume of a saturated solution of barium nitrate and two volumes of baryta-water), until a portion of the urine when taken out and filtered no longer gives a precipitate with the mixture; filter off the precipitate of barium phosphate and sulphate, wash once with water (the precipitate, after washing, may be thrown away), and evaporate the filtrate to a sirup, at first over a free flame and then when the volume amounts to about 200 cc. on the water-bath. Precipitate with about 150 cc. of alcohol, and after half an hour filter from the precipitate, which consists of salts and extractive material. Evaporate the filtrate on the water-bath as nearly to dryness as possible, and after cooling add double the volume or somewhat more of nitric acid (one part concentrated nitric acid to one part of water). The urea nitrate is filtered off (preferably next day), washed with some cold nitric acid, drained thoroughly, and dried on a porous clay plate or on filter-paper. In order to convert the urea nitrate

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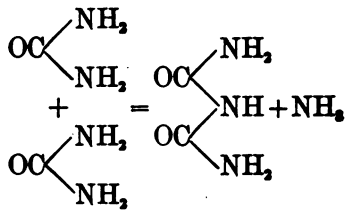
into urea, dissolve it in water in a dish, add barium carbonate, a little at a time, stirring thoroughly and heating, and continue adding the carbonate until the fluid no longer has an acid reaction, then filter, and wash once. Decolorize the filtrate, which is usually yellow-colored, by warming with some bone-black, and filter again. We must now separate the urea from the barium nitrate formed. This is done by evaporating to dryness on the water-bath and extracting the residue with alcohol, in which only the urea is soluble. The alcoholic solution is then filtered and evaporated to crystallization. The crystals of urea are left till next day, separated from the mother-liquor, pressed dry between filter-paper, and purified by recrystallizing from a small quantity of absolute alcohol (by warming in a flask).

Urea, $OC<\frac{NH_2}{NH_2}$, the amide of carbonic acid, hence also called "carbamide," crystallizes in long quadratic prisms or, when crystallized rapidly, in needles; it is very readily soluble in water (at 100° in every proportion), less readily, though still quite soluble, in alcohol, and insoluble in anhydrous ether. It is not precipitated by metallic salts, with the exception of mercuric nitrate.

Reactions of Urea.

1. Heat a small portion in a dry test-tube. It melts (melting-point 132°), giving a strong odor of ammonia, and if the urea is not dry ammonium carbonate sublimes. Continue the heating till the melted mass just begins to solidify (the re-formation of the solid is due to the change into cyanuric acid). When heated just beyond its melting-point urea forms biuret:

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Biuret gives a characteristic reaction. Dissolve the fused mass in water with the addition of some caustic soda, then add cautiously dilute copper sulphate solution; the copper hydroxide resulting dissolves, forming a reddish-violet fluid (biuret reaction).

2. Repeat the fusing of the urea, but continue the heating until the entire mass has again solidified; after cooling dissolve in water containing some caustic soda, and acidify cautiously with hydrochloric acid: precipitation of cyanuric acid, $C_3O_3N_3H_3$ (cyanic acid is first formed; this, however, polymerizes at once to cyanuric acid).

3. Dissolve a few crystals of urea on a watch-glass in a drop or two of water, and add a little concentrated oxalic acid solution: precipitation of urea oxalate $(OCN_2H_4)_2 \cdot C_2H_2O_4 + H_2O$. Examine under the microscope.

4. Repeat this experiment, using nitric acid instead of oxalic acid: urea nitrate, $OCN_2H_4 \cdot HNO_3$, (see Fig. 7).

5. Heat some urea with caustic soda solution: strong evolution of ammonia with the formation of sodium carbonate:

$$OC<\begin{matrix}NH_2\\NH_2\end{matrix} + 2NaOH = OC<\begin{matrix}ONa\\ONa\end{matrix} + 2NH_3.$$

6. Warm a small drop of mercury with nitric acid in a test-tube and then add a little urea: marked foaming due to the development of a colorless, odorless gas, a mixture of carbon dioxide and nitrogen. The reaction is due to the action of nitrous acid on urea:

$$OCN_2H_4 + N_2O_3 = CO_2 + 2N_2 + 2H_2O.$$

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7. Add to a little bromine-water in a test-tube an excess of caustic soda solution. Sodium hypobromite, NaBrO, is formed. If some urea solution is added to this mixture nitrogen is evolved, causing marked effervescence, while the



FIG. 7.—Urea Nitrate.

carbon dioxide formed at the same time is absorbed by the excess of caustic soda present:

$$OCN_2H_4 + 3NaBrO = CO_2 + N_2 + 2H_2O + 3NaBr.$$

IV. PREPARATION OF URIC ACID, $C_5H_4N_4O_3$.

(*a*) **From Urine.** Add 50 cc. of hydrochloric acid to 500 cc. of urine and let stand for twenty-four hours in a cool place, filter, and wash with water. Examine the crystals of uric acid under the microscope. Nearly neutralize the filtrate with ammonia, then add magnesia mixture, filter, and add to the filtrate some silver nitrate solution: precipitate of a double compound of silver and magnesium with that part of the uric acid which was not precipitated by the hydrochloric acid. Filter off the precipitate, wash, suspend in water, decompose by means of hydrogen sulphide, filter from the silver sulphide, evaporate to a small volume, and add hydro-

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chloric acid: precipitate of uric acid (see Quantitative Determination of Uric Acid, page 188).

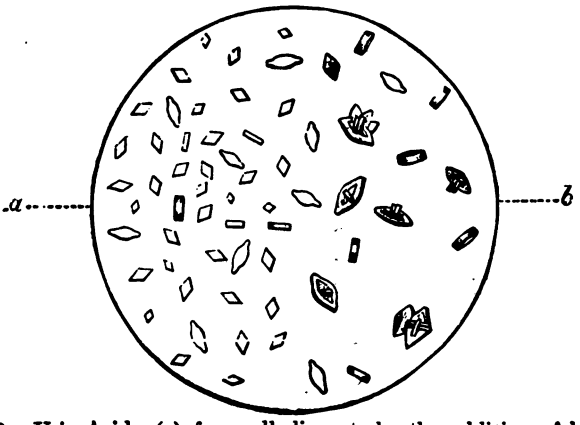


FIG. 8.—Uric Acid: (*a*) from alkali urate by the addition of hydrochloric acid; (*b*) spontaneously deposited from urine.

(*b*) **From Guano or Snake Excrement.** Heat to boiling 50 g. of finely powdered guano with 500 cc. of water and 100 cc. of caustic soda solution (marked foaming and evolution of ammonia; the experiment is therefore best performed under the hood). Keep boiling for some time, replacing the water which evaporates by hot water, until the greater part of the guano has dissolved, then filter. Pour the filtrate into about 300 cc. of dilute sulphuric acid (20 per cent.) contained in a porcelain dish, and heat to the boiling-point. Continue the heating until the fluid begins to bump and a crystalline sediment precipitates. Examine under the microscope to see that it has no amorphous sodium urate mixed with it. If it has, then more acid must be added and the heating continued with vigorous stirring or on the water-bath. Let cool, filter, wash with water until the filtrate no longer gives a precipitate with barium chloride or only a faint turbidity, drain thoroughly, and dry on filter-paper.

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If snake urine, so-called excrement, is available, 10 g. of this and 20 cc. of caustic soda solution are sufficient.

Uric acid, $C_5H_4N_4O_3$, forms a crystalline powder extremely difficultly soluble in water (in 1800 parts of hot, 14,000 [1] parts of cold water), insoluble in alcohol.

Reactions of Uric Acid.

1. Place a small quantity of uric acid with a little water on a microscope-slide and treat with some caustic soda solution or piperazine solution (10 per cent.); the crystals dissolve. When all or nearly all has dissolved add a little hydrochloric acid: the uric acid separates in characteristic spindle-shaped crystals. Examine under the microscope.

2. **Murexide Test.** Pour upon a very small quantity of uric acid on a porcelain crucible-cover some drops of nitric acid, dissolve by warming and evaporate cautiously, avoiding heating too strongly: there remains a yellow to red residue. Let cool and moisten the residue with an extremely small quantity of ammonia: purple-red color due to the formation of murexide. Now add a drop of caustic soda solution: deep-blue color. If now we heat again, the color becomes paler and disappears even before the mass becomes completely dry. If the purple-red residue or the blue residue be moistened while still hot with a few drops of water, it dissolves to an almost colorless fluid. If this is evaporated by heating with a very small flame the color is not restored, as the murexide is very easily destroyed (distinction from the reaction of the xanthine bases, especially of guanine).

3. Dissolve some uric acid in a solution of sodium carbonate and moisten with it a strip of paper which has previously been saturated with a solution of silver nitrate: spots

[1] According to His and Paul, Zeitschr. f. physiol. Chem. 31, 1 (1900–1901), 1 part dissolves in 39,500 parts of pure water at 18°.—O.

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of reduced silver appear at once. These are yellow-brown to deep black according to the quantity of the dissolved uric acid.

4. If we add to the sodium carbonate solution of uric acid some magnesia mixture (solution of magnesium hydroxide in ammonia and ammonium chloride) and then add silver nitrate solution, a double compound, silver magnesium urate, separates in the form of a gelatinous precipitate.

5. Dissolve some uric acid in water and caustic soda, add some Fehling's solution and heat: white cuprous urate precipitates, or, when the quantity of the copper relative to the uric acid is sufficiently large, red cuprous oxide is formed. This reaction is important, as it shows that, when making the Trommer's test with normal urine, the reducing action of the uric acid always present must be taken into account.

Of the reactions the precipitation of the magnesium silver urate is especially important for the isolation of uric acid, the murexide test for its detection. Uric acid is not always deposited from urine on the addition of hydrochloric acid; it is then necessary to use the precipitation of the magnesium silver compound to show its presence.

V. DETECTION OF CREATININE, $C_4H_7N_3O$.

Make 240 cc. of urine faintly alkaline by cautiously adding milk of lime and precipitate exactly with a solution of calcium chloride; dilute with water to 300 cc., mix thoroughly, and after fifteen minutes filter through a dry filter. Measure off 250 cc. of the filtrate, which must have a faintly alkaline reaction, and after neutralizing with acetic acid evaporate to about 20 cc., at first over a free flame and then on the water-bath. Mix this with the same volume of absolute alcohol, transfer the mixture to a 100-cc. measuring-flask, rinsing with absolute alcohol, and finally fill the flask up to

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the mark with the same liquid. Let stand till the next day, filter through a dry filter, and add to the filtrate about twenty drops of an alcoholic solution of zinc chloride. After standing for one or two days crystals of creatinine zinc chloride, $(C_4H_7N_3O)_2ZnCl_2$, will be deposited. Examine these under the microscope. Filter, and wash with alcohol.

For identification Weyl's reaction may be used: Grind the creatinine zinc chloride to a fine powder, boil a small quantity of this with water in a test-tube, let cool and filter. Add to the filtrate a few drops of a very dilute solution of sodium nitroprusside (freshly made) and then some caustic soda solution: deep-red color, which quickly fades to a straw-yellow.

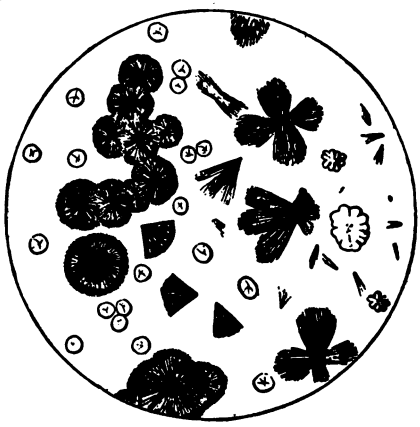


FIG. 9.—Creatinine Zinc Chloride from Urine.

The Weyl reaction may also be used directly with the urine. As the urine often contains acetone in considerable quantity and this gives a very similar test, it is advisable before trying the reaction to boil the urine for a few minutes, thus driving off the acetone and then cool.

Jaffe's reaction with picric acid may also be used directly with the urine: Add to the urine some aqueous picric acid solution and then a few drops of caustic soda: deep-red color.

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VI. Detection of Oxalic Acid.

Evaporate 500 cc. of unfiltered urine over a small free flame to about 150 cc. After cooling add 20 cc. of hydrochloric acid, transfer to a separating-funnel and shake with about an equal volume of a mixture of alcohol and ether (9 volumes of ether, 1 volume of absolute alcohol), carefully separate the ether extract and filter it through a dry filter. Repeat the extraction with ether once more and distil the combined ether extracts from a dry flask. The fluid remaining in the flask is poured into a dish, the flask being rinsed out once with a little alcohol, then with water, and the mixture is heated on the water-bath with the addition of some water until the odor of alcohol and ether has disappeared. The aqueous fluid remaining, whose volume should be about 20 cc., is cooled, and filtered from some resinous material which separates. Make the solution faintly alkaline with ammonia, add 1 or 2 cc. of a 10 per cent. calcium chloride solution, and acidify with acetic acid. The white precipitate of calcium oxalate, which forms either at once or else gradually, is amorphous, but quite homogeneous when it separates quickly. It is crystalline when precipitated more slowly, and then frequently shows, instead of octahedral forms, those described by Feser and Friedberger [1] (quadratic prisms with pyramidal end faces).

VII. Hippuric Acid, Benzoyl Glycocoll, $CH_2—NH(COC_6H_5)$ COOH

To about 300 cc. of horse-urine add milk of lime till the reaction is strongly alkaline (to separate phosphoric acid and a part of the coloring matter), filter, evaporate to sirupy

[1] Maly's Jahresber. f. Thierchemie, 4, 231.

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consistency, precipitate with alcohol, filter, evaporate the alcoholic extract and, when perfectly cold, acidify strongly with hydrochloric acid. The hippuric acid separates as a crystalline paste. Filter this off (keeping the filtrate), wash, drain by pressing between drying-paper, dissolve in water to which ammonia has been added, decolorize by boiling with some bone-black, filter, concentrate, precipitate again by adding hydrochloric acid, filter, wash and dry on drying-paper in the air. Examine a small portion while still moist under the microscope. If it proves to be contaminated with benzoic acid (irregularly indented leaflets, see Fig. 10),



FIG. 10.—*a*, benzoic acid; *b*, hippuric acid.

treat the dry mixture of acids with ether to dissolve the benzoic acid. In order to obtain larger crystals the acid may be recrystallized from hot water.

Reactions of Hippuric Acid.

1. A small portion is heated in a test-tube with water: it dissolves. On cooling the hippuric acid separates in needles. Examine under the microscope.

2. Heat a small portion in a test-tube: the hippuric acid

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melts, at first without decomposition (melting-point 187°). When heated more strongly the melted mass turns red, gives a sublimate of benzoic acid, and develops an odor resembling that of the oil of bitter almonds (benzonitrile, C_6H_5CN, and prussic acid, HCN).

The red color is due to the decomposition of the glycocoll. After the tube is cold, cut it off close below the sublimate and place the upper part of the tube in a weak solution of sodium carbonate in a test-tube. To the solution resulting add a little hydrochloric acid: precipitation of benzoic acid. Examine under the microscope.

3. Evaporate a small quantity of the crystals of hippuric acid with some drops of fuming nitric acid, mix the residue with some sand, put it into a tube and heat strongly: odor of the oil of bitter almonds due to the formation of nitrobenzene, $C_6H_5NO_2$ (Lücke's reaction). Benzoic acid and many other acids of the aromatic series also give this reaction.

VIII. PHENOL, C_6H_5OH, CRESOL, C_7H_7OH.

PHENYL SULPHURIC ACID, CRESYL SULPHURIC ACID.

(*a*) PHENOL.

Radiating crystalline mass (if previously melted) or loose crystals, forming an oily fluid with one-tenth its volume of water. Melting-point 42°; readily soluble in ether, in alcohol, and in fifteen parts of water.

Reactions of Phenol.

Use a 2 per cent. and a 0.2 per cent. solution of phenol and make parallel tests. The conduct given in the following experiments is for the 2 per cent. solution:

1. Addition of ferric chloride solution: deep blue (amethyst-blue) color. Strong acids discharge the color. Many phenol derivatives, e.g., salicylic acid, give a similar reaction with ferric chloride.

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2. Add to the phenol solution one-fourth its volume of ammonia, then a few drops of a solution of chloride of lime (bleaching-powder), and warm gently, but not to boiling: blue or green color.

3. Add a few drops of Millon's reagent and heat to boiling: intense dark-red color or dark-red precipitate. The reaction is very distinct even at 1 : 60,000, but at such great dilution only a delicate rose-color appears. All benzene derivatives which contain a hydroxyl group in the aromatic residue give a similar reaction (O. Nasse).

4. Addition of bromine-water produces at first a gelatinous precipitate of monobromphenol or dibromphenol, which is characterized by a very penetrating odor. On further addition of bromine yellowish-white tribromphenol, $C_6H_2Br_3OH$, is formed. In dilute solutions this last compound is at once formed.

Of the reactions, 3 and 4 are the most delicate and the most used.

After the medicinal use of phenol it may be found in the urine. Horse-urine and pathological urine contain cresol instead of phenol, or at least in much larger quantity. The reactions of cresol (paracresol) are similar to those of phenol, but less marked. The color with ferric chloride is not blue, but a dirty gray. This is partly due to the fact that the cresol is far less soluble in water than the phenol.

(*b*) Phenyl Sulphuric Acid or Cresyl Sulphuric Acid.

Detection of Phenol in Urine.

Phenol and cresol are never found as such in urine, but always combined with sulphuric acid in the form of the potassium salts (so-called conjugate or ethereal sulphates),

$O_2S\begin{cases}OC_6H_5\\OK\end{cases}$ or $O_2S\begin{cases}OC_7H_7\\OK\end{cases}$

Potassium phenyl sulphate Potassium cresyl sulphate

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and also in combination with glycuronic acid. To isolate the phenols or to detect their presence, these sulphuric acid esters must be decomposed. This is done by heating with hydrochloric acid.

1. **Detection in Horse-urine.** The filtrate obtained in the preparation of hippuric acid (see VII, page 105) is diluted to 200 cc., and 150 cc. distilled off. The distillate has the characteristic odor of paracresol. It frequently contains some benzoic acid in crystalline form.

A part of the cresol may be used for the reactions, the rest is made faintly alkaline with sodium carbonate solution and shaken in a separating-funnel with a little ether, the ether separated and evaporated: there remains a mixture of cresol and a little phenol.

2. **Detection in Human Urine.** Two hundred cubic centimeters of urine to which 50 cc. of hydrochloric acid have been added are distilled until a small portion of the distillate no longer gives any turbidity with bromine-water. If we wish to detect very small quantities (in normal urine), 500 cc. of the urine are first made alkaline with sodium carbonate solution and evaporated nearly to dryness, the residue treated with one-fifth of its volume of hydrochloric acid and distilled (J. Munk).

Instead of the roundabout method of distillation we may make use of a shorter procedure, which depends on the hydrolysis by nitric acid and conversion into nitro compounds. This method is used especially to decide the question whether much of the carbolic acid used in the dressing of wounds, etc., is absorbed. In this case a parallel experiment with normal urine should always be made.

Add to the urine in a test-tube some nitric acid and heat to boiling: an odor resembling that of bitter almonds becomes perceptible (formation of volatile ortho-nitrophenol); when perfectly cold add bromine-water to part of the liquid: more

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or less marked turbidity or precipitate of nitrotribromphenol. Normal urine, when treated in the same way, either remains clear or gives a faint turbidity. Make the rest of the liquid, obtained after heating with nitric acid, alkaline with caustic soda solution: orange-red color due to the formation of sodium nitrophenolate.

IX. PYROCATECHIN, $C_6H_4(OH)_2$.(o.)

Use an aqueous solution (0.1 g. : 25–50 cc.) of the commercial product for the reactions.

1. Add cautiously some dilute ferric chloride solution: green color. If now a trace of ammonia be added or, better, a trace of tartaric acid and then ammonia, the green color changes to violet. Acidifying with acetic acid restores the green color.

2. Add to some of the solution a little ammonia and then a few drops of silver nitrate solution: almost immediate reduction to metallic silver results.

3. On the addition of caustic soda solution the liquid takes up oxygen and becomes colored green, then brown and black, especially on shaking.

4. Pyrocatechin is completely precipitated by lead acetate solution, as the filtrate gives none of the above reactions.

X. INDIGO BLUE. INDICAN (POTASSIUM INDOXYL SULPHATE), $O_2S\begin{matrix}OC_8H_6N\\OK\end{matrix}$.

(*a*) Indigo Blue, $C_{16}H_{10}N_2O_2$.

1. Heat cautiously some finely powdered commercial indigo, spread out in a porcelain or metal dish. The upper surface becomes covered with purple crystals of indigo blue.

2. Heat a small portion in a dry test-tube. The tube becomes filled with a purple vapor, resembling the vapor of

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iodine. A part of the indigo carbonizes, another part sublimes; in this process, however, some of the indigo blue is always transformed into the isomeric indigo red (Rosin).

3. Heat a little indigo with some chloroform: blue solution.

4. Heat a little indigo with concentrated sulphuric acid, let cool completely, and then pour into water: blue solution of indigo mono- and di-sulphonic acids. Filter the solution and examine with the spectroscope: strong absorption-band between C and D, nearer to D.

(*b*) Detection of Indican.

1. **Jaffe's Indican Test.** Add to some of the urine an equal volume of hydrochloric acid, then, drop by drop, with constant shaking, a dilute solution of chloride of lime (bleaching-powder) (1:20). Then add about 1 cc. of chloroform and shake gently: the chloroform becomes colored blue, owing to its dissolving the indigo formed. The quantity of the chloride of lime is difficult to estimate, and an excess may oxidize the indigo blue. The reaction always take place slowly and requires some time. Urine which is rich in indican turns green or even blue directly, while urine poor in indican does not do this. Frequently, instead of a blue, a violet color results, which is not taken up by the chloroform. This is due, according to Rosin, to indigo red or to urorosein. Normal urine as a rule turns violet to red-violet, but it gives up indigo blue to the chloroform. Strongly colored urines, e.g., icteric, must be decolorized by the addition of a little basic lead acetate and filtered before making the test.

2. **Modification of the Indican Test according to Obermayer.** Precipitate the urine with the basic lead acetate solution, taking care not to use an excess, filter through a dry filter, shake the filtrate vigorously for one to two minutes with an equal volume of fuming hydrochloric acid (containing in 1000

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parts two to four parts of ferric chloride), and then with 1 cc. of chloroform. The ferric chloride is preferable to the chloride of lime, as a loss of indigo by oxidation cannot then take place.

If the urine contains alkaline iodides, then, after making the test, add a few drops of a 10 per cent. solution of sodium thiosulphate to combine with the iodine.

XI. UROBILIN.

Discovered by Jaffe in urine (febrile urine, retained urine).

Detection:

1. Some of the urine is examined with the spectroscope: urines which contain considerable urobilin often show directly the characteristic absorption-band at the border of the green and blue, between the lines b and F (see Table of Absorption Spectra, No. 7). Sometimes the absorption-band is more distinct after the addition of a few drops of hydrochloric acid. Not infrequently the absorption of light is so strong that the urine must be diluted with several times its volume of water in order to obtain a distinct absorption-band.

2. Add ammonia to a second portion of the urine, filter from the precipitated phosphates after a few minutes, and add some drops of zinc chloride solution to the filtrate: with urine containing considerable urobilin a green fluorescence becomes perceptible. The spectroscopic examination shows almost the same absorption-band (somewhat nearer to b).

3. Ten to twenty cubic centimeters of urine are acidified with a few drops of hydrochloric acid and then shaken gently with 5 to 10 cc. of amyl alcohol. Examine the amyl alcohol extract with the spectroscope. On the addition of a few drops of zinc chloride solution (1 g. zinc chloride in 100 cc. of ammoniacal alcohol) the extract shows fluorescence (Nencki and Rotschy).

4. To a fourth portion of the urine add a few cubic centi-

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meters of chloroform, mix repeatedly, avoiding shaking too vigorously, separate the chloroform, and add to it a drop of an alcoholic solution of zinc chloride; any turbidity is to be cleared up by the addition of absolute alcohol: the chloroform becomes rose-red with a greenish fluorescence (E. Wirsing [1]).

5. Urine containing a considerable quantity of urobilin gives the biuret reaction with caustic soda and copper sulphate solutions (confusion with albumoses and peptones).

If the urobilin cannot be detected by the above methods proceed as follows:

1. Precipitate 200 cc. of urine completely with basic lead acetate, filter, wash once with water, dry the precipitate at room temperature, grind it in a mortar with alcohol and 5 g. of oxalic acid, let stand twelve to twenty-four hours, and filter (if absolute alcohol is used, the drying of the precipitate may be dispensed with; it is then sufficient to drain it thoroughly on filter-paper). Make a portion of the filtrate alkaline with ammonia, filter from the ammonium oxalate which separates, and add a drop of zinc chloride solution: green fluorescence, absorption-band. Not infrequently, however, these reactions are indistinct. In this case, in order to purify the rest of the alcoholic filtrate, shake it in a separating-funnel with about 20 cc. of chloroform and enough water so that the chloroform settles readily. Separate the chloroform, filter it through a dry filter, and examine spectroscopically both before and after the addition of a drop of an alcoholic solution of zinc chloride.

The urine must not be allowed to stand too long before it is examined, as the urobilin changes on standing into a modification which lacks the most essential properties of this pigment. This takes place even with urine containing a considerable quantity of urobilin.

[1] Verhandl. d. Würzb. phys.-med. Gesellsch. N. F. 26, No. 3.

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2. Fifty cubic centimeters of urine are gently shaken with the same quantity of perfectly pure ether, which must contain no alcohol or acid; the ethereal extract is then evaporated and the residue dissolved in 2 to 3 cc. of absolute alcohol. The solution shows the green fluorescence and the absorption-band. Its color, curiously enough, is often pure yellow.

XII. Detection of Unoxidized Sulphur.

Pour some hydrochloric acid on a small piece of zinc in a dish, let the acid act for a short time, then pour off the hydrochloric acid and rinse the zinc with water. Put the zinc in a flask with about 50 cc. of urine, add enough hydrochloric acid to cause an evolution of hydrogen, and fasten a strip of filter-paper, which has been previously moistened with lead acetate solution, in the neck of the flask by means of a loosely fitting cork. The paper turns brown or black after some time from the formation of lead sulphide. Only the neutral sulphur of the urine forms hydrogen sulphide with nascent hydrogen. Neither the sulphuric acid nor the ethereal sulphates are acted on.

XIII. Detection of Pepsin.

Divide the urine to be examined, about 50 cc., into two equal parts, heat one-half to boiling, and let cool; the second half is not heated To each of the two portions add 4 or 5 drops of hydrochloric acid. Ten cubic centimeters of the urine thus prepared are placed in a test-tube with a shred of fibrin and digested at 40°. It is advisable to make two tests with each portion of the urine. The shred of fibrin dissolves in the unboiled portion of the urine in a longer or shorter time according to the amount of pepsin present, but not in the boiled portion. The unboiled portion of the urine in

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which the fibrin dissolved gives the biuret reaction (after it has been neutralized with dilute sodium carbonate solution, boiled and filtered) while the boiled portion does not.

XIV. Detection of Albumin.

1. Heat a portion of the clear, filtered urine to boiling. If it remains clear and the reaction is acid, there is no albumin present. If it becomes cloudy, the turbidity may be due to the precipitation either of albumin or of calcium phosphate. To decide this, acidify quite faintly with acetic acid: if the urine then becomes clear, the turbidity is due to calcium phosphate and the urine is free from albumin; if, on the other hand, the turbidity remains and the precipitate tends to collect in flakes on standing, the urine contains albumin. A doubt can only arise when the turbidity which remains is very small and uniform. In this case it may be due to the presence of mucin or nucleoalbumin in the urine. This is to be assumed if the urine becomes cloudy even in the cold, when acidified directly with acetic acid or after dilution with an equal volume of water. If the urine remains clear on boiling, but reacts strongly alkaline (a rare case), it may contain albumin. In this case also the addition of acetic acid to acid reaction decides the matter.

2. Instead of acetic acid we may use nitric acid to acidify the boiled urine.

3. Add to the urine one-third of its volume of nitric acid. If it remains clear (when only traces of albumin are present the turbidity appears very gradually), then it is free from albumin; if it becomes cloudy, the cloudiness may be due to albumin or to urates, albumoses, or resin acids (after the use of balsams or sandal-wood oil). If the cloudiness remains on warming, then it is due to albumin.

4. Add one-third the volume of concentrated sodium chloride solution, acidify with acetic acid to distinctly acid

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reaction, and heat to boiling. A turbidity or precipitate shows the presence of albumin (mucin remains in solution).

5. Acidify with acetic acid and add a few drops of potassium ferrocyanide solution: cloudiness proves the presence of albumin (a very delicate reaction, but only available when the urine remains clear after the addition of acetic acid alone; moreover, it is also given by albumose).

The precipitated albumin may be filtered off, washed, and used for the color reactions (see page 4).

The examination for both globulin and albumin is carried out as in the case of blood-serum (page 59), the urine being previously made alkaline with ammonia and filtered.

XV. Detection of Albumoses (Peptone).

To detect albumoses (peptone) we precipitate with phosphotungstic acid according to Hofmeister and try the biuret reaction with the precipitate, after the albumoses have been set free by means of baryta or (simpler) by caustic soda. The presence of urobilin, which is carried down in the precipitate with phosphotungstic acid and also gives the biuret reaction, complicates matters. If any considerable amount of urobilin is present, it must be removed (most readily with absolute alcohol) before trying the biuret reaction with the precipitate. When the amount of urobilin is small and the urine is not highly colored, this may be dispensed with and method 1 may be used.

1. Acidify 50 cc. of urine (for practice use urine to which 0.25 to 0.5 g. of the commercial peptone to the liter has been added) in a beaker with a few cubic centimeters of hydrochloric acid, precipitate with phosphotungstic acid, and heat on the wire gauze. In a few minutes the precipitate forms a resinous mass, which sticks to the bottom of the vessel. As soon as this has occurred, pour off the supernatant, nearly clear, liquid as completely as possible and rinse the resinous

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mass, which later becomes brittle, twice with distilled water. With some care this may easily be done almost without any loss. Pour over the precipitate a few cubic centimeters of water and dissolve it by adding sodium hydroxide solution. The deep-blue solution is warmed on the wire gauze until the color has disappeared, then poured into a test-tube, cooled, and copper sulphate solution cautiously added. The color is not always a pure violet, but often only a dirty red or even yellowish red. Often 10 to 15 cc. of the urine will give the reaction.

If the urine contains albumin it must previously be treated with sodium acetate and enough ferric chloride to give the fluid a blood-red color, the acid neutralized with dilute sodium hydroxide solution and heated to boiling. The filtrate must give neither a turbidity nor a blue color with acetic acid and potassium ferrocyanide (a very faint blue color is very difficult to avoid and may be disregarded).

If the urine contains mucin or nucleoalbumin it must be precipitated with a little neutral lead acetate solution, so that a heavy flocculent precipitate is formed. Then after filtration precipitate with phosphotungstic acid, either at once or after treatment with sodium acetate and ferric chloride in case the urine also contains albumin. In the case of animal urines the previous treatment with lead acetate solution is advisable.

2. For urines containing considerable urobilin v. Aldor[1] has modified this method as follows: 8 to 10 cc. of urine are made acid with a few drops of hydrochloric acid, precipitated with phosphotungstic acid and centrifuged. The supernatant fluid is poured off, the precipitate covered with absolute alcohol and again centrifuged. This is repeated until the alcohol remains entirely colorless, then suspend the pre-

[1] Berliner Klinische Wochenschr., 1899, Nos. 35 and 36.

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cipitate in water and dissolve by adding sodium hydroxide solution, etc. If a centrifuge is not available the precipitate may be washed with alcohol on the filter.

3. A larger amount of primary albumoses (0.3 g. commercial peptone to 100 cc. of urine) may be detected as follows: Acidify the urine with acetic acid to distinctly acid reaction and add an equal volume of concentrated salt solution: turbidity, which clears up on heating and returns on cooling.

XVI. GLUCOSE, $C_6H_{12}O_6$.

Also called grape-sugar, diabetic sugar, and dextrose, easily soluble in water and dilute alcohol, difficultly in absolute alcohol. Melting-point of the anhydrous glucose [1] 146°. The solution of glucose is dextrorotatory.

1. A small portion is cautiously heated in a dry test-tube. The glucose melts and turns yellow; when heated more strongly the melted mass turns dark brown and develops a peculiar odor—so-called caramel odor. After cooling dissolve the residue in water: deep-brown solution (caramel color used in confectionery).

2. Place a small quantity of glucose and a piece of caustic potash in a test-tube, add a few drops of water, and heat: vigorous reaction and a brown coloration. When perfectly cold acidify cautiously with dilute sulphuric acid: odor of caramel.

3. Trommer's test; 4. Bismuth test; 5. Potassium ferrocyanide test; 6. Silver test; 7. Indigo test; 8. Rubner's test; and 9. Fermentation test. See under Milk-sugar, page 11. For all these reactions use two solutions A and B, A containing 4 g. of glucose dissolved in 200 cc. of water, i.e.,

[1] Glucose also crystallizes with one molecule of water of crystallization: $C_6H_{12}O_6 + H_2O$.

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2 per cent.; and B 20 cc. of A diluted to 200 cc., i.e., 0.2 per cent.

Experiment 7 is to be made only with solution B.

10. **Phenyl Hydrazine Test.**—Dissolve by shaking 2.5 g. of phenyl hydrazine hydrochloride and 5 g. of sodium acetate in 100 cc. of a 1 per cent. glucose solution or add about 2 cc. of phenyl hydrazine, which has been previously dissolved in acetic acid to acid reaction, filter if necessary, heat for three-quarters of an hour upon the water-bath, and let cool gradually: crystalline mass composed of bright yellow needles of phenyl glucosazone, $C_{18}H_{22}N_4O_4$. Filter these off, wash, dry a small portion, and determine the melting-point. This should be 204° to 205°, if the compound is quite pure. If it is found to be considerably lower, as is frequently the case with diabetic urine, or too high, which happens also sometimes, the compound should be recrystallized from a mixture of equal volumes of water and alcohol. Most of the sugars yield osazones. These are usually characterized by their melting-points.

11. **Reaction of Molisch.**—To ten drops or 0.5 cc. of the sugar solutions (0.2 per cent. and 0.02 per cent.) add one drop of an alcoholic or methyl alcoholic solution of α-naphthol, and let 1 cc. of pure concentrated sulphuric acid cautiously run down the wall of the test-tube. At the surface of contact of the two fluids a beautiful violet-red ring will form, which, on shaking gently, increases in breadth and intensity. The reaction of Molisch is a general one and is common not only to the sugars, but to all carbohydrates.

Detection of Glucose in Urine.

Dissolve 4 g. of glucose in 100 cc. of a urine of moderate concentration (specific gravity not less than 1017): solution A. Mix 10 cc. of solution A with 90 cc. of the same urine: solution B. A part of the urine is kept for check experi-

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ments. Try the Trommer's test, the bismuth test, the silver test, and the indigo test with these solutions and with the urine itself as a check. Trommer's test is to be made both with a large and with a small quantity of copper sulphate solution.

The following experiments are to be made only with the weaker solution B and with the urine as a check:

1. **Fermentation Test.**

2. **Phenyl Hydrazine Test.** Heat 50 cc. with 2 g. of phenyl hydrazine hydrochloride and 4 g. of sodium acetate or with phenyl hydrazine acetate (see above).

3. **Reaction of Molisch** with increasing dilution in the form introduced by v. Udránszky. One drop of the urine-sugar solution B is diluted with ten drops (0.5 cc.) of water; one drop of α-naphthol solution and 1 cc. of sulphuric acid are added. Repeat in the same way with the urine as a check. Then dilute both urines and repeat the test. As a rule normal urine when diluted five times still gives a suggestion of the reaction. If the reaction appears at even greater dilution, then the amount of carbohydrate is above the normal. Since this excess need not necessarily be sugar and the limit of the reaction for the normal amount of carbohydrate is not fixed, the test is not to be regarded as conclusive.

4. **Reaction with Nylander's Bismuth Solution.** Boil 5 cc. of urine with ten drops (0.5 cc.) of the bismuth solution for some minutes. The urine-sugar solution B turns black, the urine used as a check does not. Many concentrated urines, however, turn black though they contain no sugar; this is true also of those containing chrysophanic acid.

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XVII. Examination of Diabetic Urine for Acetoacetic Acid, CH_3COCH_2COOH.

1. Add directly to some of the urine ferric chloride solution. The quantity of this should not be too small, since the iron chloride is at first used up to form ferric phosphate (gray precipitate): a red coloration indicates the presence of acetoacetic acid.

2. Acidify the urine (about 50 cc., sometimes even 10 cc. is sufficient) with dilute sulphuric acid, shake with an equal volume of ether, separate the ether and agitate it with a very little dilute ferric chloride solution. In the presence of acetoacetic acid the aqueous layer turns violet-red. After the use of salicylic acid the urine gives a very similar reaction.

XVIII. Acetone, CH_3COCH_3.

Clear colorless fluid with an agreeable odor, miscible in every proportion with water, alcohol, and ether, boiling-point 56° to 57°. To 250 cc. of urine add a few drops of acetone and some hydrochloric acid, distil off about 50 cc., and make the following tests with the distillate:

1. **The Iodoform Test.**—Add a few drops of sodium hydroxide solution, then iodine potassium iodide solution. The fluid at once becomes turbid and gives the odor of iodoform (CHI_3). On standing iodoform is deposited. Examine under the microscope. Aldehyde also gives the same reaction. The use of ammonia instead of sodium hydroxide is said to exclude this confusion (black nitrogen iodide is formed at first in this case, but gradually disappears): this test, however, is less delicate.

2. **Legal's Test.**—Add to some of the distillate enough sodium nitroprusside solution (freshly prepared) to distinctly color the fluid and then some sodium hydroxide solution: the fluid turns ruby-red. If acidified with acetic acid the

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color becomes more violet. Aldehyde gives the same reaction.

3. **Gunning's Test.**—Add to some of the distillate a few drops of mercuric chloride solution, then some sodium hydroxide solution and an equal volume of alcohol. Shake thoroughly and filter through a close filter. The filtrate must be quite clear. Acidify the filtrate faintly with hydrochloric acid and float on it some ammonium sulphide solution, so as to form two layers. At the surface of contact a grayish-black ring of mercuric sulphide will appear. The reaction depends upon the solubility of mercuric hydroxide in acetone. Aldehyde also possesses this property (v. Jaksch).

The confusion with aldehyde is not especially to be feared, since this substance has not hitherto been found in urine and could only be present if the acidified urine should be carelessly distilled too far. Aldehyde may be readily detected in the distillate by the following reactions:

1. If we add to a small portion some ammoniacal silver solution (to prepare this add to about 5 cc. of silver nitrate solution some drops of ammonia and then half the volume of sodium hydroxide solution), blackening quickly takes place.

2. If we warm a small portion after the addition of some sodium hydroxide solution, a yellow color appears (eventually turbidity also) and a characteristic odor develops (formation of aldehyde resin).

XIX. Detection of Bile-pigment.

1. **Gmelin's Test.** Float some icteric urine by means of a pipette on a few cubic centimeters of nitric acid, which contains a very small amount of nitrous acid (fuming nitric acid): colored rings where the two liquids meet and in the following order from top to bottom: green, blue, violet, red.

2. **Rosenbach's Modification of Gmelin's Test.** Filter a quantity of the urine, partly dry the filter by pressing between

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drying-paper, and moisten its inner surface with yellow nitric acid such as was used in experiment 1.

3. Make the urine alkaline with a few drops of sodium carbonate and then add, with constant shaking, drop by drop, calcium chloride solution until the supernatant fluid shows no perceptible color or only the normal urine color.

Filter off the precipitate, wash well, put it into a test-tube, pour on some alcohol, and dissolve the precipitate by adding hydrochloric acid and shaking. If the clear solution be boiled it turns green to blue in the presence of bile-pigment, and in the absence of this it remains uncolored. Let cool completely and add nitric acid. The green color changes to blue, violet, and red. According to Hammarsten a mixture of nineteen volumes of hydrochloric acid with one of nitric acid is prepared. One volume of this mixture is added to five to nine volumes of alcohol and the precipitate dissolved in this: green solution which gradually turns blue when bile-pigments are present.

The Gmelin test may be doubtful or lead to errors if the urine contains considerable indican. This is excluded by the isolation of the bile-pigment in method 3. Moreover, the test 3 often gives a positive result when test 1 does not.

XX. Detection of Dissolved Blood-pigments.[1]

Take 400 cc. of normal urine. Add to 300 cc. of this 2 cc. of diluted blood (1 : 10) and shake thoroughly. Reserve the other 100 cc. for check tests. The color of the urine to which the blood has been added would not lead us to suspect the presence of the blood-pigment.

1. **Spectroscopic Examination Direct.** If the bands of the oxyhæmoglobin are not to be seen, add to the urine, according to L. Lewin and Posner, some drops of ammonium sulphide and then a few drops of sodium hydroxide solution. The

[1] Oxyhæmoglobin and methæmoglobin.

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examination in the spectroscope then shows the very characteristic bands of reduced hæmatin (hæmochromogen of Hoppe-Seyler).

2. **Heller's Test.** Make a portion of the urine strongly alkaline with sodium hydroxide solution, heat to boiling, and let stand. The precipitate of phosphates, which collects on the bottom of the test-tube, is colored blood-red by hæmatin. Make a check experiment with normal urine.

3. Add to 100 cc. of the urine some cubic centimeters of urine containing considerable albumin, heat to boiling, collect the precipitate on a filter, and wash. Grind the precipitate in a mortar with about 20 cc. of absolute alcohol, add a few drops of concentrated sulphuric acid, heat the mixture to boiling in a flask on the water-bath, and filter. When cold make the filtrate alkaline with sodium hydroxide solution and add a few drops of ammonium sulphide. When examined with the spectroscope the fluid now shows the absorption-bands of reduced hæmatin.

4. Add to the urine a little freshly made alcoholic solution of gum guaiacum till a permanent turbidity results, then some old oil of turpentine, and shake thoroughly. On allowing to stand and shaking repeatedly the mixture or the oil of turpentine gradually becomes blue. On shaking this mixture with ether a violet color is imparted to the ether and the blue color remains in the aqueous fluid. Both gradually fade. Make a check experiment with normal urine. Not decisive in the presence of pus-cells. The latter, according to Brandenburg,[1] give the blue color even with the guaiacum tincture alone.

XXI. Detection of Hæmatoporphyrin.

1. Thirty to fifty cubic centimeters of urine containing hæmatoporphyrin are completely precipitated with an alka-

[1] Münchener medicin. Wochenschr. 1900. No. 6.

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line barium chloride solution (mixture of equal volumes of a cold saturated barium hydroxide solution and a 10 per cent. barium chloride solution), the precipitate is washed a few times with water, then once with absolute alcohol, and drained as completely as possible. Place the moist precipitate in a small mortar, add six to eight drops of hydrochloric acid, then enough absolute alcohol to make a thin paste, grind well, let stand for some time or warm gently on the water-bath, and filter through a dry filter. If the mixture gives too small a filtrate, wash with some alcohol. It is advisable, however, to prepare not more than 8 to 10 cc. of the alcoholic extract. The coloring-matter may also be extracted from the precipitate, after it has been washed with water and alcohol, by repeatedly pouring upon it a warmed mixture of about 10 cc. of absolute alcohol and six to eight drops of hydrochloric acid. The alcoholic extract is red and shows the two characteristic bands of hæmatoporphyrin in acid solution (see Table of Absorption Spectra, No. 6). If the solution is made alkaline with ammonia it takes on a yellowish shade and now shows the four absorption-bands of hæmatoporphyrin in alkaline solution.

2. Add to some of the same urine some glacial acetic acid (to 100 cc. of urine 5 cc. of glacial acetic acid) and let stand for two days; the hæmatoporphyrin is deposited as a precipitate (Nebelthau).

EXAMINATION FOR THE INORGANIC CONSTITUENTS.

I. Detection of Chlorides.

Add to the urine a few drops of nitric acid and then some silver nitrate solution; according to the amount of chlorides present in the urine there will be formed either a white precipitate of silver chloride, AgCl, which on shaking forms white cheesy flakes (normal conduct), or only a faint turbidity (febrile urine).

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II. Detection of Sulphates.

Add some hydrochloric acid to the urine and then a little barium chloride solution: white precipitate of barium sulphate, $BaSO_4$ (normal conduct), or only a faint turbidity (after the use or absorption of considerable carbolic acid).

III. Detection of Ethereal Sulphates.

Mix 20 cc. of the urine and 20 cc. of the alkaline barium chloride solution (see page 124) and filter. Boil the filtrate with fuming hydrochloric acid (one-half volume): turbidity due to the precipitation of barium sulphate, which is ordinarily slight, but if the amount of ethereal sulphates is abnormally large (from different causes) it may be considerable.

IV. Detection of Phosphates.

(*a*) **In general.** About 20 cc. of urine are treated with acetic acid and uranyl acetate solution: yellowish-white precipitate of uranium phosphate or uranyl phosphate $(UO_2)HPO_4$.

(*b*) **Separate Detection of Phosphoric Acid united to the Alkali Metals and to the Alkali-earth Metals.** Make 50 cc. of urine alkaline with ammonia and after the mixture has stood for some time filter from the precipitate of alkali-earth phosphates. The filtrate contains the phosphoric acid combined with the alkali metals, as may be shown by the addition of acetic acid and uranyl acetate solution. Dissolve the precipitate, after it has been thoroughly washed with water, by pouring on acetic acid; phosphoric acid may also be shown to be present in this solution by means of the uranyl acetate solution.

V. Detection of Ammonium Salts.

Put 25 cc. of the urine, a few drops of thymol solution, and about 25 cc. of milk of lime in the Schlösing apparatus (an

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empty desiccator may be used), and in the dish 5 cc. of water containing a few drops of hydrochloric acid to absorb the ammonia. Let stand for forty-eight hours. Show the presence of ammonia in the water in the usual manner (by means of platinum chloride or Nessler's reagent).

VI. Detection of Potassium Iodide.

Urine, voided after the use of potassium iodide, or one to which potassium iodide (0.2 per cent.) has been added, is treated with nitric acid containing some nitrous acid, a few cubic centimeters of chloroform are added and the mixture well shaken: the chloroform becomes colored violet. With urines containing considerable indican errors may arise from the formation of indigo red and indigo blue. In order to exclude these, add to the solution a little starch paste and shake thoroughly. At the surface of contact of the chloroform and the urine a ring of the blue starch-iodine product will be formed.

If the color is due to iodine it will be removed by the addition of a solution of sodium thiosulphate, while the indigo color will remain unchanged.

VII. Detection of Potassium Bromide.

Ten to twenty cubic centimeters of urine, voided after the use of potassium bromide, or one which contains 0.2 per cent. of potassium bromide, are made alkaline with sodium carbonate, about 3 g. of potassium nitrate added, evaporated to dryness in a silver or platinum dish, and then heated more strongly till the mass melts and becomes perfectly white. After cooling, dissolve the fused mass in water, acidify strongly with hydrochloric acid (not sulphuric acid), add some fresh chlorine-water, and shake with chloroform: the chloroform becomes colored yellow.

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VIII. Detection of Mercury.

Method of Fürbringer. Add to 500 cc. of urine 1 to 2 cc. of mercuric chloride solution (0.1 per cent.) and 10 cc. of hydrochloric acid. Heat to 60–80° and digest for about a quarter of an hour with 0.25 to 0.5 g. of very fine brass shavings, pour off the urine, rinse the brass shavings repeatedly with warm water, then with alcohol, and finally two or three times with ether. Then place the brass shavings, twisted into a roll, in a glass tube closed at one end, about 10 cm. long and 8 mm. wide, and draw out the tube at some distance from the brass shavings to a fine point. Heat the brass shavings gently from the bottom upwards: a sublimate forms which should be driven up to the capillary. This sublimate is by no means all mercury, but contains zinc oxide, and sometimes drops of water also condense. To detect the mercury it must be converted into the iodide. This is best done in the following manner: Cut off the under part of the tube, thus removing the roll of brass shavings, place in the upper part of the tube a grain of iodine, convert this into vapor by gently warming, and blow, with the aid of a glass tube, gently into the upper opening of the tube. The iodine vapor is thus forced over the mercury and changes this into mercuric iodide. Instead of this some iodine may be placed in the under part of the tube, which is then fused together and the iodine volatilized by heating gently. Examine the tube with a magnifying-glass or under the microscope: especially characteristic is the conversion of the yellow mercuric iodide, if it is present, into the red variety by touching it with a platinum wire, as well as the volatility of the mercuric iodide and its crystal form.

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CHAPTER XI.

EXAMINATION OF URINARY CALCULI.

HEAT a portion of the finely powdered calculus on platinum-foil; if it burns completely or leaves only a very small quantity of ash, then it consists of uric acid, ammonium urate, cystine, or xanthine. If it does not burn completely it may contain uric acid or urates, calcium phophate, and magnesium phosphate, or ammonium magnesium phosphate and calcium oxalate. The method to be pursued in the analysis is based on this difference.

I. THE POWDERED CALCULUS BURNS COMPLETELY.

Digest the powder by warming gently with dilute hydrochloric acid (1 : 2).

(*a*) **The Powder Dissolves Completely or Almost Completely.** The calculus consists of cystine or xanthine.

To test for cystine digest a small portion of the powder with ammonia, filter, let the extract evaporate on a watch-glass, and examine the residue under the microscope: cystine forms hexagonal tablets. Cystine calculi are usually small, have a yellow color and a smooth surface.

To test for xanthine make the so-called xanthine test with nitric acid and sodium hydroxide solution (see chapter on Muscular Tissue, page 29).

(*b*) **The Powder Does Not Dissolve Completely.** Filter and wash the residue.

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1. **Residue: Uric acid.** Confirm by making the murexide test (page 101). Uric acid calculi vary in size, are quite hard, and are usually colored reddish yellow or brown.

2. **Filtrate:** may contain ammonium chloride. To test for ammonia warm some of the filtrate with sodium carbonate solution: ammonia is evolved and may be detected by its odor, alkaline reaction, etc.

II. The Powder Turns Black but Does Not Burn.

A slight blackening always results when the calculi are heated, due to the presence of organic matter. A small portion of the finely powdered calculus is digested by warming with dilute hydrochloric acid (1 : 2): effervescence indicates the presence of carbonates.

(*a*) **Complete Solution.** Uric acid is not present.

(*b*) **Incomplete Solution.** The residue may contain uric acid, proteids, epithelium, etc. The general appearance or a microscopical examination usually enables us to decide what is present. The presence of the uric acid may be readily confirmed by the murexide test.

In any case the solution is to be further investigated. Warm a small portion of the filtered solution with an excess of sodium carbonate solution and test for ammonia (see above). Dilute the main quantity of the liquid with water, filter, make faintly alkaline with ammonia, cool the fluid in case it has become hot from the addition of the ammonia, and acidify with acetic acid. Either an approximately clear solution results or a turbid one, which gradually deposits a white pulverulent precipitate.

The yellowish-white flakes which are seen in the approximately clear solution consist of ferric phosphate. Prove this by filtering and dissolving the washed precipitate in hydrochloric acid: the solution is colored blue on the addition of potassium ferrocyanide.

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The white insoluble precipitate is calcium oxalate. Prove this by a microscopical examination. Filter; if the quantity is not too small, wash, dry, and heat to red heat on platinum-foil. The calcium oxalate burns to calcium carbonate and oxide. Therefore the residue, when moistened with a drop of water, shows a strong alkaline reaction and dissolves in hydrochloric acid with effervescence. The filtrate from the iron phosphate or the calcium oxalate may contain phosphoric acid, calcium, magnesium.

1. To a portion of the filtrate add some uranyl acetate solution. A yellowish-white precipitate of uranyl phosphate indicates the presence of phosphoric acid.

2. To the remainder of the filtrate add ammonium oxalate: a white precipitate indicates calcium. Heat, then filter from the precipitate, and make the filtrate alkaline with ammonia: a crystalline precipitate of ammonium magnesium phosphate indicates the presence of magnesium.

Test for ammonia by warming with sodium carbonate solution that part of the original hydrochloric acid solution which was put aside.

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CHAPTER XII.

EXAMINATION OF THE LIVER.

I. Preparation and Reactions of Glycogen.
II. Detection of Sugar.
III. Preparation of the Xanthine Bases of the Liver.

I. Preparation of Glycogen.

Introduce, by means of a tube, into the stomach of a well-fed rabbit, on the day previous to killing it, as well as five to six hours before death, 10 to 15 g. of glucose or cane-sugar dissolved in water. After killing the rabbit remove the liver. Reserve about 10 g. of this for the experiment below (detection of sugar), and chop up the rest into very small pieces. Then heat to vigorous boiling with ten times its weight of water, adding a trace of acetic acid in order to facilitate the precipitation of the proteids. Filter the extract, which shows a marked opalescence, through muslin, press the residue thoroughly, grind it in a mortar, and boil again with water, filter, and press out the residue as before. Evaporate the united extracts to about 100 to 150 cc., acidify with hydrochloric acid, and add Brücke's solution [1] (potassium mercuric iodide), then alternately a few drops of hydrochloric acid and Brücke's solution until the precipitation is complete. The addition of the Brücke's solution is for the purpose of pre-

[1] Made as follows: To a hot 5 to 10 per cent. solution of potassium iodide add with constant stirring mercuric iodide until a portion remains undissolved, let cool, and then filter.

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cipitating the proteids still present in the solution and also the gelatine formed on boiling. Now filter, wash once with water, add double the volume of 90 per cent. alcohol, and stir thoroughly. After the precipitate has settled completely filter and wash, first with a mixture of two volumes of alcohol and one volume of water, then with absolute alcohol, and finally with ether; or, in case it is somewhat voluminous, it is better to remove it from the filter, grind with absolute alcohol, let stand some time with this, filter, press out the alcohol, and treat in the same manner with ether. Finally the glycogen is freed from the adhering ether by pressing and by grinding in a mortar.[1] The method of preparation of S. Fränkel [2] is also very convenient: Grind the liver, without heating it, with 2.5 times the quantity of a 2 to 4 per cent. solution of trichloracetic acid, filter, wash with some of the trichloracetic acid solution, and precipitate with alcohol, etc. The trichloracetic acid has the property of coagulating the proteids and completely precipitating them.

Thus prepared glycogen, $C_6H_{10}O_5$ (according to Huppert $6(C_6H_{10}O_5)+H_2O$), is a chalky-white fine powder in which hard transparent pieces resembling gum arabic may be present in case of insufficient dehydration; it dissolves readily, though somewhat slowly, in water, always forming an opalescent solution, which is extremely strongly dextrorotatory (according to E. Külz α_j is $+211°$, according to Huppert α_D is $+196.63°$). On boiling with acids it forms glycogen-dextrin and then glucose; when treated with saliva or pancreas extract it is converted into glycogen-dextrin and maltose. It forms, like other carbohydrates, oxalic acid when boiled with nitric acid. It is distinguished by its characteristic conduct towards iodine solution.

[1] For a better method for the preparation of glycogen see Salkowski, Zeit. f. Physiol. Chem. 36 (257). See also page 131 of this book.—O.

[2] Pflüger's Archiv, 52, 125.

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Reactions of Glycogen.

1. Heat a small portion of the substance on platinum-foil until all the carbon is burned: only a very small amount of ash should remain.

2. Dissolve 0.25 g. by warming with 50 cc. of water or 0.5 g. in 100 cc.

(*a*) Determination of the rotation: If the dextrorotation is not distinctly apparent, add some sodium hydroxide solution.

(*b*) Add to a small portion of the solution a very small quantity of iodine potassium iodide solution saturated with sodium chloride. The solution turns reddish-brown; continue to add the iodine as long as the intensity of the color perceptibly increases, then divide the mixture into two parts. Heat one part gently: the color disappears, but returns on cooling. The addition of sodium hydroxide decolorizes the solution at once, sodium carbonate acts more slowly (combination with the iodine), acids gradually decolorize the solution (forming sugar).

According to Hoppe-Seyler, in order to determine the presence of glycogen in neutral solutions, place equal portions of dilute iodine solution in two test-tubes of the same diameter, to one add some of the solution to be tested, to the other the same quantity of water, and compare the colors.

(*c*) Dissolve some commercial peptone in a few cubic centimeters of the glycogen solution and make the test with iodine: the reaction only appears after the addition of considerable iodine and may not appear at all when the quantity of the peptone exceeds that of the glycogen. The color formed frequently disappears on standing from the gradual combination of the iodine. Impure, dilute solutions of glycogen, therefore, give a poor iodine reaction.

(*d*) Boil a few cubic centimeters of the glycogen solution a short time with about one-third of its volume of hydrochloric

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acid: the opalescence disappears owing to the conversion of the glycogen into glycogen-dextrin and glucose. Neutralize the cold mixture with sodium hydroxide or make it faintly alkaline, add a few drops of Fehling's solution and heat: precipitation of red cuprous oxide.

(*e*) Digest a few cubic centimeters of the glycogen solution with about 1 cc. of saliva at 40°: in a very short time the opalescence disappears and the solution no longer gives the iodine reaction; if the digestion be continued one to two hours the solution will give a marked reaction for sugar (formation of maltose).

(*f*) Add to a few cubic centimeters of the solution a drop or two of lead acetate and then pass in hydrogen sulphide: deep-black fluid, from which no lead sulphide separates and which is not changed by filtering; glycogen, like gelatin, has the property of holding fine precipitates in suspension, though not to such an extent.

II. Detection of Sugar.

That part of the liver which was reserved for showing the presence of sugar is kept for twenty-four hours and is then chopped up. Heat it to boiling with ten times its weight of water, adding a trace of acetic acid to precipitate the proteids, filter, evaporate to about one-fifth of its volume, filter again and use the solution for the reactions for the detection of glucose (see chapter on Urine, page 117). Trommer's test, the fermentation test, and the phenylhydrazine test are sufficient.

III. Preparation of the Xanthine Bases.

Chop up finely 250 g. of beef- or calf's liver, put it into a 3.5 to 4 liter stoppered bottle with 2.5 liters of chloroform-water,[1] then add about 2.5 cc. more of chloroform, shake vigorously

[1] Made by shaking vigorously 2.5 liters of water with 12.5 cc. of chloroform in a glass-stoppered bottle till the chloroform has dissolved.

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several times, then digest two to three days in an air-bath at 40°. The digestion with chloroform-water effects two objects: (1) it causes the complete cleavage of the nucleins; and (2) it removes substances, always present in the extracts of organs, which disturb or entirely prevent the precipitation of the xanthine bases by silver nitrate. The first object can only be attained otherwise by boiling with dilute acids (Kossel). Whether the second may also be completely effected by dilute acids alone has not been determined as yet with certainty, though it is probable. At any rate, the digestion with chloroform-water has the advantage of great convenience.

After the digestion heat the mixture to boiling in a large enameled iron dish or agate-ware pan and continue the boiling, after acidifying faintly with acetic acid, until the albumin has been completely precipitated. Then filter and evaporate further to a volume of 800 to 1000 cc. Make the solution moderately alkaline with ammonia, filter from the slight precipitate formed, and precipitate completely with a 3 per cent. ammoniacal solution of silver nitrate: a gelatinous precipitate of the silver compounds of the xanthine bases is formed. Care must be taken that no silver chloride precipitates. If this should happen, more ammonia must be added. Add the silver nitrate solution until a small portion of the filtrate, after acidifying with nitric acid and adding some hydrochloric acid, gives a milky turbidity. Filter off the precipitate, wash well, place it in a flask and dissolve it while still moist in hot nitric acid of 1.1 specific gravity (equal volumes of nitric acid, specific gravity 1.2, and water) to which some urea has been added. The solution must be almost clear. Filter hot and let stand twenty-four hours. Guanine-, adenine- and hypoxanthine-silver nitrate crystallize out, while xanthine-silver nitrate remains in solution. Filter and wash the precipitate.

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(*a*) Make the filtrate alkaline with ammonia: precipitate of xanthine-silver. For further treatment see chapter on Muscular Tissue, page 25.

(*b*) Suspend the precipitate of guanine-, adenine- and hypoxanthine-silver nitrate in water and decompose by passing in hydrogen sulphide, filter from the silver sulphide, evaporate to a small volume, make alkaline with ammonia, and let stand. Guanine crystallizes out, while hypoxanthine and adenine (besides ammonium nitrate) remain in solution. Filter, wash a few times, and use the guanine for the reactions.

Guanine, $C_5H_5N_5O$ (amino-hypoxanthine, $C_5H_3N_4O(NH_2)$), is insoluble in water, soluble in sodium and potassium hydroxide solutions and also in acids forming salts. It is distinguished from the other xanthine bases by being almost insoluble in ammonia. Of all the xanthine bases it gives the strongest so-called xanthine reaction and, like xanthine, gives a reaction with hypochlorites. Like hypoxanthine and adenine the compound with silver nitrate is insoluble in dilute nitric acid.

Reactions of Guanine.

1. Try the xanthine reaction (see chapter on Muscular Tissue, page 29). The residue left after evaporating the nitric acid solution turns intensely dark red, even bluish red on moistening with sodium hydroxide solution.

2. Dissolve a small portion in hydrochloric acid and add a little saturated aqueous solution of picric acid: a crystalline precipitate gradually forms.

3. Mix in a watch-glass some sodium hydroxide solution with a little chloride of lime and put into the mixture a grain of guanine; around this there will be formed a dark-green ring. This color soon turns to brown and then gradually disappears. Xanthine gives the same reaction (originally given by Hoppe-Seyler for xanthine).

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CHAPTER XIII.

EXAMINATION OF BONE.

Pour 10 cc. of water upon some pieces of hollow bone (about 3 g.) in a beaker, add 10 cc. of hydrochloric acid (evolution of carbon dioxide follows the addition of the acid), and let stand at room temperature for twenty-four hours. The dilute hydrochloric acid dissolves the inorganic constituents of the bone and leaves the ossein, which retains the shape of the original bone, undissolved.

I. Ossein and Gelatin (Glutin).

Pour off the hydrochloric acid solution and preserve for further investigation (under III). Wash the ossein several times with water, then let it lie in water containing a few drops of sodium carbonate solution and again wash with water. Place it in a beaker with a small quantity of water, heat this to boiling, and continue the boiling until the pieces of ossein have for the most part dissolved (five to ten minutes)[1]. Neutralize or make the solution faintly alkaline with sodium carbonate solution, decant into a test-tube, and place this in cold water. After some time the solution will form a more or less firm jelly of bone-gelatin (also called glutin). The ossein is converted into gelatin or glutin by boiling with water.

[1] Frequently there remains an inner part of the bone, which was not attacked by the acid.

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II. Conduct of Gelatin (Glutin).

Use a solution of commercial gelatin (the best white gelatin) for the following experiments.

About 5 g. of gelatin are covered with water in an evaporating-dish and allowed to stand. On the next day or after several hours it will appear much swollen, but not dissolved. The supernatant fluid is poured off, 40 cc. of water added, the mixture heated on the water-bath until the gelatin is dissolved, and then cooled: very soon a tolerably firm jelly will be obtained. To this add 190 cc. more of water and warm again. The solution thus obtained, about 2 per cent., is to be used for the following reactions after it has cooled somewhat:

1. Portions of the solution are treated in test-tubes with (*a*) tannin solution, and also with (*b*) hydrochloric and phosphotungstic acids: voluminous precipitates. General conduct of all proteids, their immediate derivatives (albumoses and peptones), and also of the albuminoids.

2. Boiling the solution produces no precipitate even when acetic acid is added.

3. The addition of acetic acid and potassium ferrocyanide produces no precipitate (distinction from albumin and albumoses); under certain conditions, however, a precipitate may be formed (Mörner).[1]

4. The addition of mercuric chloride gives no precipitate (distinction from albumoses and peptone).

5. Boiling after the addition of one-third of its volume of nitric acid produces only a very faint yellow color; gelatin yields only extremely little so-called xanthoproteic acid, as the aromatic group is almost entirely lacking in the molecule, and the phenol or tyrosine group especially is absent.

6. The addition of sodium hydroxide and some copper

[1] Zeitschr. f. physiol. Chem. 28, 489.

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sulphate solution gives a blue-violet color, which, however, never shows a purple-red shade (distinction from peptone); on heating to boiling the color becomes more red, if but little copper sulphate solution has been added; if much copper sulphate has been added, boiling produces no perceptible color change.

7. Boiling with Millon's reagent produces only a faint rose or red coloration (it is advisable to first heat the gelatin solution to boiling, add a *few* drops of Millon's reagent, and then heat again). Distinction from albumin, due to the absence of the tyrosine group in the molecule. The slight red coloration is to be attributed to admixture with albumoses or peptone.[1]

8. The addition of bromine-water produces a voluminous yellow precipitate of a viscous sticky nature.

Gelatin possesses in the highest degree the property of holding many precipitates in the state of the finest suspension, so that they pass through all filters, or it may even prevent entirely the formation of precipitates.

(*a*) Add to a portion of the gelatin solution in a test-tube a drop or two of basic lead acetate: the solution remains unchanged (gelatin is not precipitated by metallic salts in general). Now dilute the mixture to about 30 cc. and pass in hydrogen sulphide: there results a brownish-black fluid, which passes through filter-paper unchanged; dilute a portion of this with water: a clear light-brown solution results from which no lead sulphide separates.

(*b*) Dissolve a very small quantity of hypoxanthine in a few cubic centimeters of dilute ammonia, divide the solution into two approximately equal parts, and add to the one (1) double the volume of water, to the other (2) double the volume of the gelatin solution. To both portions add silver

[1] According to Van Name *pure* gelatin gives the Millon's reaction, see Jour. of Expr. Med. 1897, 11, 117.—O.

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nitrate solution: (1) yields a flocculent precipitate of hypoxanthine-silver, (2) does not, the solution only becomes faintly opalescent. The gelatin prevents the precipitation of the hypoxanthine-silver completely.

III. The Mineral Constituents of Bone.

Make half of the hydrochloric acid solution alkaline with ammonia, then acidify with acetic acid: the precipitate formed dissolves on the addition of ammonia, leaving a slight residue of ferric phosphate, which presumably comes from the blood contained in the bone (at least in part from this). Filter and use a small part of the filtrate to test for phosphoric acid, the greater part to test for calcium and magnesium.

(*a*) The flocculent precipitate is washed and dissolved in a few cubic centimeters of dilute hydrochloric acid. Test for iron in the solution with potassium ferrocyanide, and for phosphoric acid with ammonium molybdate.

(*b*) Filtrate from the ferric phosphate.

1. Test for phosphoric acid by adding uranyl acetate solution: yellowish-white precipitate of uranyl phosphate $(UO_2)HPO_4$.

2. Precipitate the calcium as calcium oxalate, $CaC_2O_4+H_2O$, by the addition of sufficient ammonium oxalate. To the clear filtrate (made clear by warming and pouring repeatedly through the filter), which must remain clear on the further addition of ammonium oxalate, add ammonia to alkaline reaction: a crystalline precipitate of ammonium magnesium phosphate, $MgNH_4PO_4+6H_2O$, separates after some minutes.

The detection of the mineral constituents of bone may also be made with the bone-ash. For this purpose 0.5 to 1 g. of the bone-ash is sufficient. The carbonates are more easily recognized by this method. The course of the examination is the same.

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CHAPTER XIV.

EXAMINATION OF ADIPOSE TISSUE.

I. Separation of Fat and Connective Tissue.
II. Decomposition of Fat into Fatty Acids and Glycerin.

I. SEPARATION OF FAT AND CONNECTIVE TISSUE.

Cut into fine pieces with a knife or a pair of shears 10 g. of adipose tissue,[1] grind as finely as possible in a mortar, place in a flask, and heat to boiling on a water-bath with 40 cc. of absolute alcohol. The fat dissolves, leaving the connective tissue behind. Filter, wash, first with alcohol, then once or twice with ether, press the residue on the filter between filter-paper, and allow the ether still adhering to evaporate by letting it lie in the air: fibrous mass consisting of fat-cells and connective tissue. Show the presence of albumin in the residue by heating with nitric acid and then adding sodium hydroxide solution (xanthoproteic reaction), and also by boiling some of the substance with water and adding a few drops of Millon's reagent. The conversion of the connective tissue into gelatin cannot be brought about by simply boiling with water as in the case of ossein. A higher temperature (boiling under pressure) or very long-continued boiling is required to accomplish this.

[1] Preferably unsmoked hog-fat.

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On cautious evaporation on the water-bath, the ethereal-alcoholic solution yields fat, which slowly solidifies.

Reactions of Fat.

1. Rub a small portion of the fat on paper (not filter-paper). The paper becomes transparent.

2. Add to a few cubic centimeters of alcohol one to two drops of very dilute sodium hydroxide solution (about tenth-normal caustic soda solution containing 0.4 per cent of NaOH), then add enough rosolic acid solution or phenolphthaleïn solution to make the fluid intensely red. At the same time dissolve a little fat (one drop or a piece the size of a pea) in a few cubic centimeters of ether and pour the ethereal solution of the fat into the solution of the indicator. The solution does not change its red color, the fat reacts neutral.

3. Grind in a mortar a small quantity (one drop or a piece the size of a pea) with some powdered potassium bisulphate (monopotassium sulphate) and heat the mixture in a dry test-tube: penetrating odor (caution!) of acroleïn (acrylic aldehyde, $CH_2=CH-CHO$). A strip of filter-paper moistened with an ammoniacal silver nitrate solution (see Acetone, page 121) turns black immediately (reduction to silver) when placed in the upper part of the tube. The fat is here decomposed and the glycerin converted into acroleïn by the elimination of water.

4. Warm a small portion of the fat in a test-tube with sodium carbonate solution: the fat forms a temporary emulsion, but does not dissolve; saponification does not take place. Sodium hydroxide solution also does not saponify fat at room temperatures.

II. DECOMPOSITION OF FAT, SAPONIFICATION.

When heated with caustic potash or caustic soda (very readily in alcoholic solution) the fats are decomposed with

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the assumption of the elements of water, into fatty acids, which combine with the alkalies to form soaps and glycerin; for example,

$$\underset{\text{Tripalmitin}}{C_{51}H_{98}O_6} + \underset{\text{Water}}{3H_2O} = 3\underset{\text{Palmitic Acid}}{(C_{16}H_{32}O_2)} + \underset{\text{Glycerin}}{C_3H_8O_3}.$$

Process of Saponification.

Weigh off about 15 g. of caustic potash in an evaporating-dish, add 10 cc. of water, and heat on the water-bath until the caustic potash has dissolved. At the same time make up 100 cc. of 90 per cent. (volume per cent.) alcohol in a measuring-cylinder. Pour the caustic potash solution into a 400-cc. flask and rinse out the dish with a part of the alcohol. Then weigh off 50 g. of lard in an evaporating-dish, place the dish on the water-bath, heat till the fat is completely melted, pour the melted fat into the same flask, wash out the fat remaining in the dish by heating with portions of the alcohol on the water-bath, and finally pour the rest of the alcohol into the flask. Place the flask on a hot-water bath, heat, and cautiously shake the contents thoroughly as soon as the alcohol begins to boil. Saponification takes place very quickly, almost immediately.[1] In order to determine with certainty whether the saponification is completed, pour a small quantity of the alcoholic fluid into a little distilled water: the solution must be clear; it should contain no unsaponified fat in the form of drops of oil. The solution will then contain soap and glycerin besides the excess of caustic potash and the alcohol.

Separation of the Fatty Acids and the Glycerin.

Pour the contents of the flask gradually and with constant stirring into hot dilute sulphuric acid contained in a

[1] If we heat the alcoholic solutions of the fat and caustic potash to boiling separately and pour the two solutions together, saponification does take place at once on shaking.

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beaker. The sulphuric acid must be somewhat more than equivalent to the caustic potash used (12 g. of concentrated sulphuric acid poured into 250 cc. of water or 60 cc. of 20 per cent. sulphuric acid [1] and 200 cc. of water). The fatty acids separate as an oily layer. When all of the soap solution has been added, let cool or cool in water, break up the layer of fatty acids, pour off the aqueous fluid and preserve for further examination for glycerin. Break up the fatty acids into small pieces with a glass rod, place them on the filter and wash with distilled water until the wash-water no longer gives any reaction for sulphuric acid.[2] Then put the fatty acids in an evaporating-dish, place this on the water-bath, and heat till they are melted. Let cool perfectly, and free the cake of fatty acids thus obtained from the adhering water by means of absorbent paper. These fatty acids form a mixture of oleic acid, $C_{18}H_{34}O_2$ (fluid fatty acid), palmitic acid, $C_{16}H_{32}O_2$, and stearic acid, $C_{18}H_{36}O_2$ (solid fatty acids).

Reactions with Small Quantities of the Fatty Acids.

1. Action on paper, same as in the case of fat.

2. Conduct towards the alkaline solution of rosolic acid or phenolphthaleïn. The rosolic acid solution turns yellow, and the phenolphthaleïn solution becomes colorless. Even a fairly large amount of tenth-normal sodium hydroxide solution may be added without restoring the red color: the fatty acids react acid.

3. Conduct on heating with monopotassium sulphate: no acroleïn is formed.

[1] By 20 per cent. sulphuric acid is always meant one that contains 200 grams of concentrated sulphuric acid in one liter.

[2] The fatty acids thus prepared are not perfectly pure. They always contain some potassium sulphate and some soap. If it is desired to have the fatty acids free from these substances, they must either be repeatedly melted with water, or, more simply, extracted with ether, the ether extract shaken with water, and the ethereal solution distilled or evaporated.

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4. Conduct on heating with a half-saturated sodium carbonate solution: the fatty acids dissolve, carbon dioxide is evolved, and a sodium soap is formed. Cool the test-tube in water: the solution solidifies to a jelly of so-called soap gelatin.

5. Pour 100 cc. of water upon 2 g. of fatty acids, heat and dissolve the fatty acids by neutralizing with a solution of sodium hydroxide: soap solution. The following reactions are to be made with small portions of this solution while it is still warm.

(*a*) Addition of hydrochloric acid: precipitation of fatty acids.

(*b*) Addition of calcium chloride solution: insoluble calcium soap is formed and the solution loses the property of foaming when shaken.

(*c*) Addition of lead acetate: white precipitate, which becomes viscous and sticky on warming; lead plaster.

(*d*) Pour on a few cubic centimeters of the soap solution some drops of a vegetable oil or cod-liver oil and shake once or twice: homogeneous milky fluid due to the formation of an emulsion. The soaps possess in a high degree the property of emulsifying fats. Repeat the last experiment, using, however, instead of the soap solution, four drops of sodium carbonate solution; emulsion often takes place in this case also, but only when the fat contains free fatty acids, for then soap is formed from the fatty acids and the sodium carbonate. Absolutely neutral fats containing no free fatty acids are not emulsified when treated with sodium carbonate solution.

(*e*) Place some fatty acids in a dry test-tube, and in another tube about the same amount of fat. Place both tubes in a beaker partly filled with water, heat the beaker on the wire gauze, stirring the water constantly with a glass rod (having a piece of rubber tubing on its end), in order to

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obtain the most even distribution of temperature possible: the fat melts sooner than the corresponding fatty acid, i.e., the melting-point of the first is lower. This is an invariable rule.

Separation of the Solid Fatty Acids from Oleic Acid.

Heat the remainder of the fatty acids in a beaker on the water-bath till melted, then add 100 cc. of 70 per cent. alcohol, continue heating somewhat longer, filter while hot into a dish or beaker, and let cool completely. A paste of crystallized solid fatty acids forms, while the oleic acid, together with a part of the solid fatty acids, remain in solution. Dilute the pasty mass with 200 cc. of 70 per cent. alcohol, filter through a dry filter, wash with some 70 per cent. alcohol, and preserve the filtrate. Press the solid fatty acids dry between filter-paper. The filtrate, when evaporated on the water-bath, yields, when cold, a salve-like mass consisting of oleic acid mixed with some solid fatty acids.

The preparation of pure oleic acid as well as the separation of palmitic from stearic acid require somewhat more detailed methods of procedure.

Preparation of Oleic Acid.

The semi-solid fatty acids are dissolved by heating with sodium carbonate solution and a considerable quantity of water (clear solution), and neutral lead acetate is added to the solution as long as a precipitate forms. The mixture is then faintly acidified with acetic acid. The lead salts separate in viscous lumpy masses. Pour off the supernatant fluid, knead the lead salts with warm water, decant and remove the water adhering by heating on the water-bath. When cold break up the lead plaster into small pieces, grind it with about three times its volume of gypsum or caolin (slightly burnt and ground clay) or sand, place the mixture

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in a dry flask, pour on two to three times the volume of ether, and let stand, shaking repeatedly, till next day. Precipitate the lead completely from the filtered ethereal solution by means of hydrochloric acid, place the ethereal solution in a separating-funnel, and shake repeatedly with water. The solution, when separated and filtered through a dry filter, yields oleic acid on distillation or evaporation of the ether. The purification of the oleic acid depends upon the solubility of the lead oleate in ether and the insolubility of the lead palmitate and stearate. Some of the lead palmitate and stearate always dissolves, however, and some of the oleate remains undissolved in the residue.

Separation of Palmitic from Stearic Acid.

Dissolve the solid fatty acids in 95 per cent. alcohol (to each gram 20 cc. of alcohol), then take a tenth of the solution and determine how much of an alcoholic solution of neutral lead acetate is necessary for complete precipitation. Measure off nine times as much of the same lead acetate solution and divide it into five equal parts. Add the first fifth to the alcoholic solution of the fatty acids, filter, then precipitate with the second fifth, etc. (fractional precipitation). Each precipitate is washed with cold alcohol, pressed between filter-paper, decomposed with hydrochloric acid, and extracted with ether. The ether extract is washed with water, the ether evaporated, and the melting-point [1] of the residue determined. A complete separation of palmitic from stearic acid is only to be attained by repeating the fractional precipitation several times. That the solid fatty acids form a mixture of different acids may also be shown in a very simple way by allowing a hot solution of 5 g. of the fatty acids in 100 cc. of 95 per cent. alcohol to stand till next day, filtering off the fatty acids

[1] Sheep-fat gives more solid fatty acids, especially stearic acid, than hog-fat.

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which crystallize, pressing out the mother-liquor between filter-paper and, when dry, determining the melting-point: this should be about 66°. By evaporating the alcoholic mother-liquor an acid having a melting-point of 56° will be obtained. Both preparations are mixtures of palmitic and stearic acids. In the first stearic acid is in excess, in the second palmitic acid.

Separation of Glycerin, $C_3H_5(OH)_3$.

The aqueous solution obtained from the soap solution by precipitating the fatty acids contains glycerin, besides potassium sulphate and free sulphuric acid. Filter, nearly neutralize with sodium hydroxide solution, and then completely neutralize with sodium carbonate solution, evaporate, as nearly to dryness as possible, at first over a free flame, then on the water-bath, and mix the residue with 50 cc. of 90 per cent. alcohol. Filter the solution after it has stood for some time, evaporate again on the water-bath as completely as possible, and dissolve the residue in absolute alcohol, so that the volume of the mixture amounts to 25 cc. in all. Without filtering add 25 cc. of ether, shake thoroughly, and let stand for some time, preferably till next day. The ether precipitates the greater part of the remaining salts. Filter and evaporate the ethereal-alcoholic filtrate cautiously by gently warming on a water-bath. The glycerin is obtained in the form of a light yellow-colored sirup having an intensely sweet taste.

Reactions of Glycerin.

1. Mix in a watch-glass a drop of glycerin with a little borax and heat the mixture on a platinum wire in the Bunsen flame. The flame is colored green for a short time (formation of the glycerin ester of boric acid).[1]

[1] A better method of performing this experiment is to mix a little borax and glycerin in a small porcelain dish or crucible, heat gently with

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2. Try the acroleïn test with a few drops of the glycerin (see page 142).

3. Dilute the rest of the glycerin with water (clear solution), add a little sodium hydroxide and then a few drops of copper sulphate. The cupric hydroxide, which first separates, dissolves, forming a deep-blue fluid. This solution does not give any cuprous oxide on heating, but remains unchanged (distinction from many sugars, especially glucose).

a small Bunsen flame for a few minutes, add a little alcohol, and ignite. The alcohol burns with a yellow flame (due to the sodium in the borax) in which the green color (due to the boric acid glycerin ester) may be plainly seen. The object of heating the glycerin and borax previous to the addition of the alcohol is for the purpose of forming the glycerin borate.—O.

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CHAPTER XV.

YOLK AND WHITE OF THE EGG.

(*a*) Yolk of the Egg.

I. Separation of the Yolk into its Constituents.
II. Preparation of Vitellin; Detection of Lecithin.

(*b*) White of the Egg.

I. Reactions of Egg-albumin.
II. Detection of Glucose in the White of the Egg.
III. Preparation of Ovomucoid.

(*a*) YOLK OF THE EGG.

I. Separation of the Yolk into its Constituents.

Shake the yolks of several hen's eggs with ether.

- **Ether extract A:** reserve a small quantity, distil the rest; boil the residue with barium hydroxide solution.
 - **Insoluble residue B:** treat with ether.
 - **Ether extract C:** cholesterin, luteïn.
 - **Residue D:** barium soaps.
 - **Solution E:** treat with CO_2 and filter; contains glycerin-phosphoric acid, choline and glycerin.
- **Residue F:** digest with gastric juice.
 - **Solution G:** albumoses and peptone.
 - **Residue H:** paranuclein or paranucleic acid.

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Shake vigorously the yolks of ten hen's eggs in a flask or in a wide-necked separating-funnel with two to three times their volume of ether and draw off the yellow-colored ether solution as soon as it has thoroughly separated. If this separation does not take place readily, add a little alcohol. Filter the ether solution through a dry filter and repeat the extraction with fresh portions of ether until the ether has only a faint yellow color. The amount of ether used may be considerably lessened if the successive ether extracts are distilled and the distilled ether used over again. A small portion of the first ether extract is reserved for the reactions.

The main quantity of the ether extract A is distilled, the residue, while still hot, is removed from the flask to an evaporating-dish, preferably an enameled iron dish, the flask rinsed with small quantities of ether, and the ether removed by evaporation on the water-bath. Boil the residue persistently with 50 g. of crystallized barium hydroxide and 400 cc. of water, replacing from time to time the water which evaporates. In this process the fat is split up into fatty acids, which form insoluble barium soaps, and glycerin; the lecithin into fatty acids (barium soaps), glycerin-phosphoric acid, and choline.

The complete saponification is attained with difficulty and yet it is very desirable for the further work. It is best to remove from the aqueous fluid the sticky lumps of barium soaps which separate and which contain much undecomposed substance, extract them with ether, evaporate the ether extract, and boil the residue again with barium hydroxide solution. The decomposition is complete when a portion of the ether extract of the barium soaps, after evaporating the ether, no longer gives the acrolein reaction (see chapter on Adipose Tissue, page 142).

Finally the barium soaps are removed from the aqueous fluid by filtration and washed.

The crude barium soap B is freed from water as far as

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possible by heating in an evaporating-dish (and also in part by pouring off the water), then, when it is perfectly cold, it is broken up as finely as possible, placed in a flask, treated several times with ether until it is almost entirely decolorized, and then filtered.

The ether extract C is distilled; on standing the residue yields cholesterin, which is pressed between filter-paper and identified by the chloroform sulphuric acid reaction (see chapter on Biliary Calculi, page 91). The paper, which was used to free the cholesterin from its mother-liquor, is extracted with ether, and this is examined with the spectroscope (luteïn). When the treatment with baryta-water is long continued, however, the luteïn is frequently so changed that it cannot be detected in this way with certainty. It is therefore advisable to use the reserved part of the original ether solution for its detection. When this is examined spectroscopically the blue of the spectrum appears completely absorbed; on diluting with ether to the proper point an absorption-band between the green and the blue appears, and also a suggestion of a second band in the blue. A portion of the ether extract quickly becomes colorless when nitric acid is added, after giving a transient green color. Evaporate the remainder of the reserved part of the ether solution on the water-bath and dissolve the residue in a little chloroform.

Shake a part of the chloroform solution with a dilute solution of sodium hydroxide: the coloring matter is not withdrawn from the chloroform by the alkali (distinction from bilirubin or hæmatoidin).

To another part of the chloroform solution add some strong nitric acid and shake: at first a blue color and then decolorization.

The residue D is ground in a mortar with an excess of hydrochloric acid, the pasty mass placed in a separating-

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funnel, some more water added, and the mixture extracted with ether. Separate the aqueous solution containing barium chloride, and wash the ether extract several times with water. The ether leaves, on evaporating, fatty acids (in regard to the methods of identifying these see chapter on Adipose Tissue, page 144).

The solution E is freed from the excess of barium hydroxide by passing in carbon dioxide and filtering from the barium carbonate. The filtrate contains glycerin, glycerin-phosphoric acid, and choline. It is evaporated on the water-bath as completely as possible. To show the presence of the glycerin-phosphoric acid, $C_3H_5(OH)_2PO_4H_2$, grind a part of this residue with several times its volume of the oxidizing mixture, heat in a crucible till fused, and show the presence of phosphoric acid in the fused mass by means of ammonium molybdate (see chapter on Milk, page 9). Since barium phosphate is insoluble in water, the detection of phosphoric acid in this case proves the presence of an acid containing phosphorus, which forms a soluble barium salt. Such an acid is glycerin-phosphoric acid.

To detect the choline, $\left.\begin{matrix} HOCH_2.CH_2 \\ (CH_3)_3 \end{matrix}\right\}$ NOH, extract the greater part of the residue resulting from the evaporation of solution E with absolute alcohol,[1] precipitate the solution with platinum chloride, filter off the precipitate, wash with alcohol, and crystallize the choline platinum chloride from water. This crystallizes in large orange-red prisms or hexagonal plates.

The residue F is freed from ether by grinding in a mortar

[1] Barium glycerin-phosphate remains undissolved, but a considerable portion of the glycerin-phosphoric acid always passes into solution, presumably as choline glycerin-phosphate, as the choline acts like ammonium carbonate in the presence of carbonic acid, i.e., it precipitates barium carbonate from the barium glycerin-phosphate.

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and then digested twenty-four to forty-eight hours with one liter of artificial gastric juice (see chapter on Digestion, page 40). The albumin passes into solution as acid albumin, albumose, and peptone, and an insoluble residue of paranucleïn or a mixture of paranucleïn and paranucleic acid [1] remains. Filter this off, wash with water, alcohol, and ether, and show the presence of phosphorus by fusing with soda and saltpeter (see chapter on Milk, page 9).

II. Preparation of Vitellin, Detection of Lecithin Direct.

Shake vigorously the yolks of two fresh eggs in a wide-necked glass-stoppered vessel with 200 cc. of pure ether which is free from acid, and then add 5 cc. of alcohol. The addition of the alcohol causes a viscous, slimy precipitate [2] to settle from the turbid mixture. Pour off the ether solution as completely as possible and add 100 cc. of a 15 per cent. sodium chloride solution to the precipitate. On shaking the precipitate dissolves in the salt solution, forming a somewhat turbid fluid; place the fluid in a separating-funnel and shake it with an equal volume of ether. It will then become almost clear. Separate the aqueous fluid and let it stand till next day; usually the fluid becomes turbid again. Remove this turbidity by shaking again with ether. Draw off the aqueous fluid again, measure it and pour it into ten times its volume of water. Filter off the precipitate, which forms, next day, and wash with water and then with alcohol. In this condition the precipitate will contain a considerable quantity of

[1] This residue sometimes contains a very large amount of phosphorus, as much as 9 per cent.

[2] Frequently a precipitate forms even on shaking with ether alone; this precipitate is always flocculent and is insoluble in sodium chloride solution. When this phenomenon is observed further work with the material is useless. Presumably the age of the eggs has some influence on their conduct.

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lecithin, but it is not known whether this is chemically combined with the nucleoalbumin, vitellin, or only adheres to it. Place the precipitate in a flask and boil on the water-bath with absolute alcohol, filter, wash with alcohol, then with ether, and finally grind in a mortar or place in a vacuum desiccator over sulphuric acid to remove the ether. A fine white or light-yellow powder, which contains only 0.95 per cent. of phosphorus, is obtained. Its solubility is essentially different from that of the first precipitate, which still contains lecithin; presumably the vitellin is coagulated by boiling with alcohol. No method is yet known by which the vitellin may be freed from lecithin without coagulating it.[1]

The alcoholic solution yields on evaporation on the water-bath a yellow viscous residue which consists essentially of lecithin.

(*b*) THE WHITE OF THE EGG.

The albumen or white of the egg consists principally of a concentrated solution (about 11 to 12 per cent.) of a specific proteid, ovalbumin or egg-albumin, which is enclosed in a network of membranes much less in quantity. Besides the egg-albumin there are also present very small quantities of a globulin, also ovomucoid, glucose, and inorganic salts.

I. Reactions of Egg-Albumin.

For these reactions shake vigorously 20 cc. of albumen with 150 cc. of water in a flask and then filter. The solution (about 1.5 per cent.) must be clear or only faintly opalescent. The first portions of the filtrate are frequently turbid; these

[1] It might be supposed that vitellin could be prepared in connection with the separation of the yolk of egg into its constituents given under I, but it has been found that this is generally not advantageous, as the residue left when large quantities are worked up does not as a rule dissolve easily in sodium chloride solution; presumably this is due to the length of the treatment.

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are poured back on the filter till they run through clear. Test the reaction and repeat the experiments described under blood-serum (see chapter on Blood, page 60). The reactions of egg-albumin are very similar to those of the dilute serum; they differ from them, however, in some particulars.

1. On heating to boiling, the solution becomes milky and the turbidity is more pronounced than in the case of serum solution; a separation of coagulated albumin, however, does not take place. The cautious addition of acetic acid brings about the coagulation. The precipitate is not so flocculent as in the case of serum albumin, but appears somewhat swollen. A further addition of acetic acid dissolves the precipitate, but not so readily as in the case of serum albumin.[1] Repeat experiments 2, 3, and 4, under Blood-serum, with the egg-albumin solution.

5. Heat a portion of the solution with half its volume of sodium hydroxide solution: alkali albuminate is formed. Neutralize the cooled solution with dilute sulphuric or acetic acid: the albuminate precipitates. In excess of the acid this dissolves far more difficultly than in the case of serum albumin.

6 and 7 as with the serum.

8. If a portion of the solution is treated with nitric acid till a permanent precipitate is formed and then absolute alcohol is added till the volume has been doubled, the precipitated albumin does not dissolve, or dissolves only very slightly (distinction from serum albumin).

9. If strong nitric acid of the specific gravity 1.48 be added to a little of the solution, a precipitate is formed which does not dissolve when the quantity of the nitric acid added amounts to about half of the volume of the albumin solution

[1] The expression serum albumin is here used only for the sake of brevity in place of "proteids of the blood-serum."

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used. In order to dissolve the precipitate a much greater addition of acid or heating of the solution is necessary (distinction from serum albumin).

10. On shaking a small quantity of the solution with an equal volume of ether, coagulation gradually takes place.

11. On heating a small portion of the solution after the addition of an equal volume of sodium hydroxide of 1.34 specific gravity and about three drops of neutral lead acetate, it turns black. The blackening is more pronounced than in the case of serum albumin. If this solution is then acidified with hydrochloric acid there results, not a turbid grayish-yellow fluid as in the case of serum albumin, but coarse dark-gray flakes precipitate, while the fluid becomes almost clear. This distinction is due to the fact, first, that more sulphur is split off from the egg-albumin, and, secondly, that the albuminate from the egg-albumin dissolves with more difficulty in hydrochloric acid than the albuminate from serum albumin.

The reactions of a solution ten times as dilute agree entirely with those given for the correspondingly dilute serum.

II. Detection of Glucose.

Shake up the white of a hen's egg with ten times its volume of water (about 200 cc.), add acetic acid to neutral reaction, and heat to vigorous boiling in a large evaporating-dish over a free flame and with constant stirring (caution on account of the strong foaming) until the albumin has separated in lumps and the fluid appears quite clear. Then filter, wash with some water, and evaporate the filtrate and the wash-water over a free flame to a small volume, about 10 to 12 cc. Use half of this for the Trommer's test with sodium hydroxide and copper sulphate solutions, and the other half for the fermentation test. Both give positive results.

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III. Preparation of Ovomucoid.

There is present in the albumen of the hen's egg, besides the ovalbumin, a mucin-like substance, in considerable quantity (about one-eighth of the organic part of the dry substance). This does not coagulate and is characterized by its very peculiar physical conduct.

In order to prepare it, add to the albumen of three hen's eggs four times the volume of water, shake thoroughly, filter, pour the filtrate into 1.5 times the volume of boiling water, add acetic acid to neutral or very slightly acid reaction, and heat with constant stirring over a free flame to vigorous boiling. Filter, evaporate the filtrate (which should give no precipitate with mercuric chloride solution) at first over a free flame and then on the water-bath to about 20 cc. Filter the solution again, if necessary, and pour it into 100 cc. of absolute alcohol, filter, wash the precipitate once with ordinary alcohol, then once with absolute alcohol, finally with ether, and dry by allowing the ether to evaporate in the air.

The ovomucoid thus obtained, which forms a fine white powder, is dissolved in 100 cc. of water and the solution divided into three equal parts.

1. Evaporate one part of the solution to dryness on the water-bath: a substance resembling horn is left. When this is covered with water and allowed to stand it swells and forms a jelly-like mass.

2. Mercuric chloride solution gives no precipitate; but a solution of tannin and also phosphotungstic acid with hydrochloric acid give precipitates. Millon's reaction is also positive.

3. To the third part of the solution add 7 to 8 cc. of hydrochloric acid, heat to boiling, keep boiling gently for five minutes, let cool, neutralize and make the test for sugar according to Trommer and also with the freshly mixed Fehling's solution. The separation of the cuprous oxide as a rule takes place only after cooling or allowing to stand.

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CHAPTER XVI.

EXAMINATION OF THE PRODUCTS OF THE PUTREFACTION OF PROTEIDS.

I. ABRIDGED METHOD.

Five hundred grams of chopped meat, 2 liters of water, and 60 cc. of a cold saturated solution of sodium carbonate are placed in a bottle, thoroughly mixed by shaking, and digested at 40° for 6 to 8 days; the bottle being loosely closed by means of a plug of cotton. When the time given has elapsed, subject the entire mass to distillation without the addition of acid. When the contents of the distilling-flask or retort become somewhat thick, let cool, add another liter of water, and distil again. The distillate and residue are worked up separately.

(a) Treatment of the Distillate.[1]

The distillate is acidified with hydrochloric acid and extracted with ether.

- Ether solution (A) shaken with sodium hydroxide solution.
 - Ether solution (C) evaporated: indol and also skatol.
 - Alkaline solution (D) acidified, sodium carbonate solution added, then extracted with ether.
 - Ether solution (E) evaporated: phenol and cresol.
 - Alkaline fluid (F) contains the sodium salts of the volatile fatty acids (and some phenylpropionic acid).
- Aqueous fluid (B) evaporated.

[1] Zeitschr. f. physiol. Chemie, 9, 492.

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The extraction of the first distillate is best done in a separating-funnel and in separate portions of about 300 cc. with 200 cc. of ether. After shaking vigorously draw off the aqueous fluid (B) and pour into the separating-funnel a new quantity of the distillate, etc. Since the ether is dissolved to some extent by water, some fresh ether is to be added each time.

When the entire distillate has been extracted, place all the aqueous fluid (B) in a large dish and let it stand until the dissolved ether has volatilized spontaneously, then evaporate: there remains a white mass of salts, which consist very largely of ammonium chloride.

The ether solution (A) is now very thoroughly shaken with the same volume of water and 50 cc. of sodium hydroxide solution. The volatile acids formed in the putrefaction are dissolved in the alkaline solution (D), while indol and also skatol remain in the ether solution. Distil off most of the ether from the ether extract (now designated (C)) at a gentle heat on the water-bath, and let the residue evaporate spontaneously. Impure indol remains. For the reactions of this substance see page 169.

The alkaline solution (D) is again acidified with hydrochloric acid, sodium carbonate solution added until the fluid reacts acid only from the carbonic acid or is neutral (a portion taken out and heated must show an alkaline reaction when cold), and the mixture shaken with ether. Phenol and cresol are dissolved by the ether, while the volatile fatty acids remain in the aqueous fluid as alkali salts. As carbon dioxide is evolved on shaking, considerable pressure develops in the separating-funnel; it is therefore necessary to be cautious and to remove the stopper repeatedly or to reverse the separating-funnel and open the stop-cock. The ether extract (E) is then separated from the aqueous fluid (F). The ethereal solution (E), when evaporated, leaves an impure

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mixture of phenol and cresol, principally paracresol. In order to show the presence of these substances, heat the oil in a flask with some water and let cool.

1. Add some ferric chloride to a portion of the solution: dirty bluish-gray color.

2. Warm a second portion of the solution with some Millon's reagent: red color.

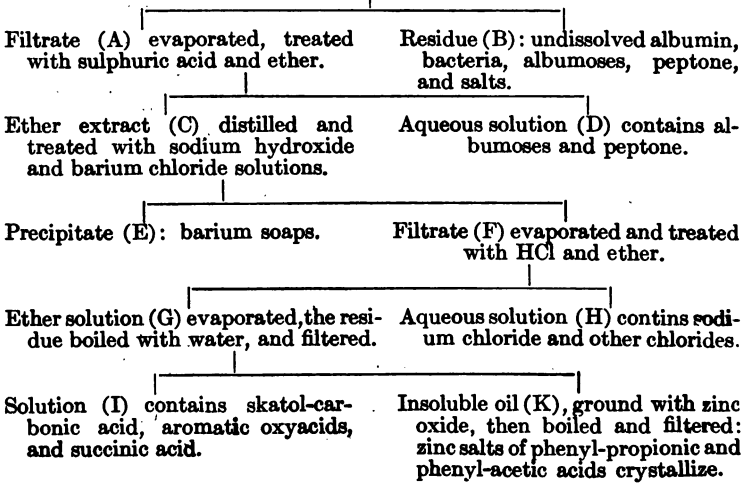
3. Add to a third portion some bromine-water; precipitate of tribromphenol and tribromcresol (or other bromine compounds).

For more detailed information concerning phenol and cresol see pages 106 and 171.

The aqueous fluid (F) is again placed in the separating-funnel, strongly acidified with hydrochloric acid, and extracted with a small quantity of ether (caution on account of the carbon dioxide set free). The ethereal solution when drawn off and evaporated leaves the volatile fatty acids, mixed with a small quantity of the homologues of benzoic acid.

(*b*) Treatment of the Distillation Residue.

The distillation residue is evaporated, precipitated with alcohol, and filtered.



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The distillation residue has an acid reaction. In order to avoid the escape of phenyl-propionic and phenyl-acetic acid with the steam on evaporating, it must be made alkaline with sodium carbonate solution. Since the fluid still contains ammonium salts, it must be again made alkaline from time to time. Evaporate to a sirup, precipitate with several times its volume of alcohol, and filter, best not till next day, from the precipitate (B) consisting of undissolved albumin, etc.

The filtrate (A) is freed from alcohol by evaporating on the water-bath, the residue dissolved in 150 cc. of dilute sulphuric acid (20 per cent, i.e., 200 g. H_2SO_4 in the liter), and repeatedly, but not too violently, shaken with ether. The ether frequently settles very slowly, and it is often necessary to add alcohol to facilitate the separation of the ether.

The aqueous solution (D) remaining contains, besides free sulphuric acid, albumoses and peptone.

The ether extract (C) is distilled, the residue dissolved in water and sodium hydroxide solution (added to alkaline reaction), and, in order to precipitate the palmitic, stearic, and oleic acids, barium chloride solution is added as long as a precipitate forms, and after allowing to stand awhile the liquid is filtered.

The precipitate (E) consists of barium soaps.

The filtrate (F) is evaporated to about 100 cc., placed in a separating-funnel, 100 cc. of hydrochloric acid added, and extracted with ether.

The ether solution (G) is drawn off, distilled, and then evaporated on the water-bath. The oil remaining is washed into a flask with hot water, boiled with about 100 cc. of water, cooled and filtered.

The aqueous solution (H) contains sodium chloride and hydrochlorides of some organic bases. To isolate these, evaporate the solution as completely as possible and extract the residue with absolute alcohol. After allowing to stand for some time, filter the solution and evaporate to dryness on

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the water-bath. Extract the residue again with absolute alcohol and continue this treatment until the residue dissolves completely in absolute alcohol, forming a clear solution. The residue of hydrochlorides—principally of δ-aminovaleric acid,[1] $C_5H_{11}NO_2HCl$,—which remains on evaporation, solidifies gradually when meat has been used, but when fibrin and gelatin have been used a crystalline mass results immediately on cooling.

The aqueous solution (I) contains skatol-carbonic acid and oxyacids, as may be proved by their reactions; the skatol-carbonic acid by its reaction with ferric chloride, the oxyacids by their conduct with Millon's reagent and with bromine-water (see further page 172).

The oil (K) insoluble in water is ground in a mortar with zinc oxide, the mixture washed into a flask with water and heated to boiling. Filter while hot. From the filtrate a zinc salt very soon crystallizes; usually this is a mixture of the zinc salts of phenyl-propionic and phenyl-acetic acids.

II. More Detailed Examination.

Place two kilos of blood-fibrin in a large flask with eight liters of water, add 2 g. of potassium phosphate (KH_2PO_4), 1 g. of crystallized magnesium sulphate, 200–240 cc. of a cold saturated solution of sodium carbonate, and then add some macerated meat to start the putrefaction. This macerated meat is obtained by allowing a mixture of 10 g. of finely chopped meat, 100 cc. of water, and 1–2 cc. of sodium carbonate solution to stand for twenty-four hours at 40–42°. Add a few cubic centimeters of this mixture as well as some of the solid particles to the contents of the flask. The flask is then closed with a cork carrying a glass tube which is connected, by means of a rubber tube, with a wash-bottle containing a 3 per cent. solution of mercuric cyanide. By this means

[1] See H. Salkowski, Ber. d. d. chem. Ges. 31, 776.

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the methyl mercaptan, discovered by Nencki among the gases formed during putrefaction, is collected in the form of the mercury mercaptide. This arrangement also helps materially to diminish the odor of putrefaction in the room in which the experiment takes place.

Digest about six days or even longer and then distil the mixture, best from a large metal vessel. Measure the distillate. As soon as seven liters have distilled over pour about 2.5 liters of water into the distillation vessel and distil off the same amount. The indol, skatol, and phenol pass over almost completely with the strongly ammoniacal distillate. The distillate also contains—besides hydrogen sulphide or ammonium sulphide, ammonium carbonate and ammonium bases—small quantities of volatile fatty and aromatic acids, while the greater part of these acids remains in the distillation residue as the sodium salts. The method of treatment of the distillate and of the distillation residue, apart from some slight modifications, is the same as given above, except that the isolation or purification of the products can be carried further on account of the larger quantities of the substances at command.

(*a*) Distillate.

1. To get rid of the disagreeable hydrogen sulphide it is advisable to add some copper sulphate solution to the distillate and to filter from the copper sulphide. The aqueous fluid (B) (see scheme on page 159) is evaporated as before, but, since it contains copper sulphate, it is advantageous to distil the residue with sodium hydroxide solution, collecting the ammonia and the other bases in hydrochloric acid, and then evaporate the solution thus obtained. If no copper sulphate has been used, this roundabout way may be dispensed with. To isolate the ammonium bases, the residue resulting is extracted in the usual manner with absolute alco-

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hol, which leaves the ammonium chloride undissolved, and the ammonium bases are precipitated with platinum chloride, etc.

2. The indol obtained by evaporating the ether solution (C) is still impure, containing especially phenol or cresol. In order to remove these impurities, wash the mixture into a flask with hot water, add sodium hydroxide solution, and distil, preferably in a current of steam. The indol passes over into the receiver, partly as a half-melted white mass, partly in the form of leaflets and some deposits as a solid in the condenser-tube. To remove the indol from this to the receiver after all the indol has distilled, it is best to attach a flask containing ether to the condenser in place of the distilling-flask and gently warm it on the water-bath. The ether which condenses in the condenser-tube dissolves the indol, and the ethereal solution runs into the receiver; finally, the whole of the indol is extracted from the distillate with ether, the ether solution separated, and, after distilling off most of the ether, allowed to evaporate spontaneously. The alkaline fluid remaining in the distilling-flask is added to the alkaline solution (D).

Very frequently the indol thus obtained contains skatol. To detect this substance, it is sufficient, when it is present in any considerable quantity, to distil a portion of the indol with water: the first drops of the distillate will contain principally skatol in the form of leaflets with a mother-of-pearl lustre, as the skatol is much more readily volatile with steam than indol.[1]

3. The crude mixture of phenol and cresol obtained by evaporating the ether solution (E) is also to be purified by distillation in steam after it has been made alkaline with sodium carbonate solution. A small portion of these substances is lost in this process. From the distillate the substances in question are again extracted with ether.

[1] Zeitschr. f. phys. Chemie, 8, 438.

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4. The acids obtained from the alkaline fluid (F) are to be combined with the volatile acids obtained from the distillation residue (see further below).

(*b*) Distillation Residue.

On account of the larger quantity of the albuminous material used, the quantity of the reagents is also to be correspondingly increased (about four times). The more detailed treatment refers especially to the acids obtained from the distillation residue. Some other modifications which facilitate the purification of the individual products of putrefaction are, however, also advantageous.

The following scheme is a good one to use:[1]

Treatment of the Distillation Residue.

The residue is made alkaline with sodium carbonate, evaporated, and precipitated with alcohol.

- Precipitate (A) (undissolved albumin, bacteria, and salts).
- Alcoholic solution evaporated, acidified with sulphuric acid, and extracted with ether.
 - Ether extract distilled, made alkaline with NaOH,[2] extracted with ether.
 - Ether extract (C).
 - Alkaline solution precipitated with $BaCl_2$ and filtered.
 - Filtrate treated with HCl and ether.
 - Acid aqueous solution (E) basic substances.
 - Ether extract (F) evaporated and distilled with steam.
 - Volatile: fatty acids, homologues of benzoic acid; collected in sodium hydroxide solution, HCl added, and extracted with ether.
 - Non-volatile: oxyacids, skatolcarbonic acid, succinic acid.
 - Precipitate (D) barium soaps.
 - Aqueous solution (B) contains H_2SO_4, peptone, and basic substances.

[1] The examination for ptomaïnes is not taken up in the following scheme. In this connection the reader is referred to "Brieger: Investigations on the Ptomaïnes," Berlin, 1885–1886.

[2] The required quantity of sodium hydroxide solution is measured off.

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REMARKS.—The oily residue remaining after distilling the ether extract (F), which contains volatlie acids, oxyacids, skatol-carbonic acid, and succinic acid, is placed in a flask and distilled in a rapid current of superheated steam. The steam is superheated by passing it through a *gently* heated copper spiral.[1] This should not be heated too strongly, as the skatol-carbonic acid will then be resinified to a considerable extent. This resinification cannot be entirely avoided, however, in any case. The vapors are conducted directly into sodium hydroxide solution, which, of course, becomes quite hot.

It is well not to have the current of steam too strong at first, as then too much of the acid escapes unabsorbed. The previous treatment of the distillation residue gives sufficient data to estimate the quantity of sodium hydroxide solution to be placed in the receiver; use about the quantity which was needed to make the first acid ether extract alkaline. To drive over the volatile acids completely requires considerable time, twenty-four to thirty-six hours. As a criterion we make use of the conduct of a very weak alkaline fluid (containing 1 to 2 cc. one-tenth normal sodium hydroxide), placed in a receiver: if this fluid still remains alkaline after an hour, the distillation is to be regarded as completed.

The entire alkaline solution is evaporated on the water-bath and, after it is cold, strongly acidified[2] with hydrochloric acid and extracted with ether. The residue left after distilling the ether extract is distilled from a flask provided with a thermometer. The volatile fatty acids distil first. The receiver is changed when the temperature has risen to about 260° and the distillation is continued until only a very

[1] Zeitschr. f. physiol. Chemie. 9, 493

[2] To detect the presence of free hydrochloric acid test the reaction of a few drops of the fluid, after the first extraction with ether, with methyl violet.

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slight residue remains in the flask. A mixture of phenyl-acetic acid and phenyl-propionic acid is thus obtained, from which frequently, but not always, one of the two acids separates.

A quantitative method for the separation of these two acids is not known at the present time. To detect the two we may either make use of their conduct in the animal body—phenyl-propionic acid is converted into hippuric acid, phenyl-acetic acid into phenaceturic acid, which are readily separated [1]—or of one of the other methods given.[2] If it is only desired to prove the presence of the homologues of benzoic acid, Lücke's reaction is sufficient (see page 106).

The solution left in the distilling-flask, after driving out the volatile acids, which contains skatol-carbonic acid, oxy-acids, and succinic acid, gradually becomes turbid on cooling and deposits some resinous substance. It must be filtered as soon as this material has settled so that filtration is possible (after some hours). From the clear filtrate there deposits after twenty-four hours' standing in the cold, best in the ice-chest, chalky-white grains of pure skatol-carbonic acid. By evaporating the aqueous solution, separated from the skatol-carbonic acid, to half of its volume, a new deposit of skatol-carbonic acid is frequently obtained; the separation is, however, never complete, a portion of the skatol-carbonic acid always remaining in the aqueous solution, together with the oxyacids and succinic acid. The complete separation of the oxyacids from succinic acid has also not yet been accomplished. If the aqueous solution be shaken with pure ether, the oxyacids, together with the skatol-carbonic acid still present, are extracted, but some of the succinic acid is also taken up by the ether, while the greater part remains in the

[1] Zeitschr. f. physiol. Chemie, **9**, 503.

[2] Ibid. **10**, 150, and H. Salkowski, Ber. d. d. chem. Ges., **18**, 323.

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aqueous solution. The aromatic oxyacids may be obtained by crystallizing from hot water the residue remaining after evaporating the ether solution.

To separate the two acids, the hydroparacumaric acid and the paraoxyphenyl-acetic acid, their conduct towards benzene may be utilized according to E. Baumann. Both acids are difficultly soluble in benzene; the hydroparacumaric acid is, however, more readily soluble than the paraoxyphenyl-acetic acid. A quantitative method of separation is not yet known.

PROPERTIES AND REACTIONS OF THE PRODUCTS OBTAINED.

1. **Indol,** $C_6H_4\langle\begin{matrix}CH\\NH\end{matrix}\rangle CH$.

Indol crystallizes from hot water in shining white leaflets, and is easily volatile with steam. Melting-point 52°. It is difficultly soluble in water, readily soluble in ether, alcohol, benzene, and chloroform. If a solution of picric acid in benzene be added to a solution of indol in petroleum ether, shining red needles of a compound of equal molecules of indol and picric acid precipitate. These when distilled with ammonia yield indol.—Indol, when introduced into the animal body, is oxidized to indoxyl and appears in the urine as potassium-indoxyl sulphate (indican), $O_2S\langle\begin{matrix}OC_8H_6N\\OK\end{matrix}$.

REACTIONS OF INDOL.

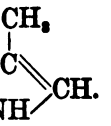
(*a*) If a cold saturated aqueous solution of indol be acidified with nitric acid and then a few drops of potassium nitrite be added, a flocculent bright brick-red precipitate of nitroso-indol nitrate, according to Nencki, $C_{16}H_{13}(NO)N_2.HNO_3$, is formed. Very dilute solutions of indol only turn red; if shaken with chloroform, a red-colored crust separates at the surface of contact of the chloroform with the aqueous fluid.

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(*b*) Legal's Reaction. Add a few drops of sodium nitroprusside solution (freshly prepared) till a distinct yellow color appears, and then some drops of sodium hydroxide solution: deep violet color. On acidifying with acetic acid the fluid turns azure-blue.

(*c*) So-called "Cholera-red Reaction." Very dilute indol solutions, which also contain a nitrite, give a splendid purple color with concentrated sulphuric acid. Cultures of the cholera bacilli also give this color, as they contain both indol and a nitrite. The nature of the coloring matter is not known. To perform the reaction [1] add to 10 cc. of a very dilute indol solution (containing 0.03 to 0 05 part per thousand) 1 cc. of a 0.02 per cent. potassium nitrite solution, mix thoroughly, and pour in some concentrated sulphuric acid, so that it forms a layer on the bottom of the test-tube: purple color. On neutralizing with sodium hydroxide solution the fluid turns blue-green. The reaction also takes place on mixing with dilute sulphuric acid. When used with cultures it is only to be regarded as proving the presence of cholera bacilli and some other kinds of bacilli, when the sulphuric acid used is absolutely free from nitrous acid.

2. Skatol, Methyl Indol, C_9H_9N



Colorless, shining leaflets, more readily volatile with steam than indol, of a penetrating fecal odor, which is scarcely perceptible when the skatol is pure. Melting-point 95°. More difficultly soluble in water than indol, readily soluble in ether, alcohol, chloroform, and benzene. When introduced into the organism it is oxidized to skatoxyl, which appears in the urine as potassium skatoxyl sulphate, $O_2S\langle^{OC_9H_8N}_{OK}$, (Brieger).

[1] E. Salkowski; Virchow's Archiv, **110**, 366 (1887).

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REACTIONS OF SKATOL.

(*a*) Skatol dissolves in concentrated hydrochloric acid with a violet color.

(*b*) If the aqueous solution be acidified with nitric acid and then a few drops of potassium nitrite solution be added, no red color results, as in the case of indol, but only a white turbidity.

3. Phenol.

For the properties and reactions of this substance see chapter on Urine, page 106.

4. Paracresol, $C_6H_4\langle^{CH_3}_{OH}\ (p)$.

This substance occurs mixed with other cresols (ortho- and meta-) in coal-tar. In its general properties it resembles phenol very closely, though it melts at a lower temperature (36°) and is far more difficultly soluble in water than phenol. It is a stronger antiseptic than phenol and is less poisonous. When introduced into the animal organism it appears in the urine for the most part as paracresyl-sulphuric acid (which also occurs in horse-urine), in part also as paraoxy-benzoic acid. In addition to the paracresol small quantities of the other cresols are also formed in the putrefaction of proteids.

The reactions of paracresol in aqueous solution are very similar to those of phenol. The color with ferric chloride solution is, however, not pure blue, but a dirty grayish blue.

5. Phenyl-acetic Acid, $C_6H_5CH_2CO_2H$.

This substance crystallizes in large, extremely thin leaflets which melt at 76.5°. It is readily soluble in alcohol, ether, and hot water, but only slightly soluble in cold water. On oxidation with potassium bichromate and sulphuric acid it yields benzoic acid. In the organism it is converted into

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phenaceturic acid, $(C_6H_5CH_2CO)NHCH_2COOH$, which appears in the urine and is found constantly in horse-urine together with hippuric acid. Phenyl-acetic acid has no characteristic reactions. It gives the Lücke's reaction with nitric acid like benzoic acid. The greater solubility of its zinc salt [1] and its conduct in the organism distinguishes it from phenyl-propionic acid.

6. Phenyl-propionic Acid, Hydrocinnamic Acid, $C_6H_5CH_2CH_2COOH$.

Phenyl-propionic acid or hydrocinnamic acid crystallizes in long fine needles melting at 48.5°, and like phenyl-acetic acid it yields benzoic acid on heating with potassium bichromate and sulphuric acid. The solubilities of the acid are about the same as those of phenyl-acetic acid; the zinc salt is very difficultly soluble. In the organism phenyl-propionic acid is oxidized to benzoic acid, which appears as hippuric acid in the urine. Phenyl-propionic acid is normally the first product in the formation of hippuric acid.

7. Paroxyphenyl-acetic Acid, $C_6H_4\begin{cases}OH\ (p)\\CH_2COOH\end{cases}$.

This substance crystallizes from water in prismatic, extremely brittle needles, usually flat, which melt at 148°. It is fairly soluble in cold water, readily soluble in hot water, alcohol, and ether, more difficultly soluble in benzene. The aqueous solution gives with ferric chloride solution a faint color, at first gray-violet, then dirty gray. It reacts positively with Millon's reagent, and also gives a turbidity or precipitate with bromine-water. When introduced into the organism it is for the most part excreted unchanged. A part is converted into oxyphenaceturic acid.

[1] Zeitschr. f. physiol. Chemie, 10, 150.

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8. Hydroparacumaric Acid, Paroxyphenyl-propionic Acid, $C_6H_4\begin{matrix}OH\\CH_2CH_2CO_2H\end{matrix}$ (*p*).

This substance is very similar in its properties to the previous acid, but is more readily soluble in water and in benzene. Melting-point 127°. According to Baumann it also occurs in the urine, together with paroxyphenyl-acetic acid.

9. Skatol-carbonic Acid, $C_9H_8N.COOH$.

Skatol-carbonic acid crystallizes in leaflets which are readily soluble in alcohol and ether, less in hot water, still less in cold water, and difficultly in benzene. Melting-point 164°. When heated beyond its melting-point it decomposes into skatol and carbon dioxide. When introduced into the organism it is excreted unchanged.

REACTIONS OF THE AQUEOUS SOLUTION (1 : 1000).[1]

(*a*) If a few drops of pure nitric acid of 1.2 specific gravity be added to the solution and then some drops of a 2 per cent. solution of potassium nitrite, it turns cherry-red after a time, then becomes turbid, depositing a red coloring matter which is not identical with the nitrosoindol nitrate.

(*b*) If an equal volume of hydrochloric acid (of 1.12 sp. gr.) be added to the solution and then a few drops of a weak (1–2 per cent.) solution of chloride of lime, it gradually turns purple-red and deposits a purple-red precipitate.

(*c*) If some drops of hydrochloric acid and a few drops of very dilute ferric chloride are added to the solution and the mixture heated, it turns cherry-red even before the temperature reaches the boiling-point.

[1] Zeitschr. f. physiol. Chemie, **9**, 24.

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10. Succinic Acid, $\begin{matrix} CH_2COOH \\ | \\ CH_2COOH \end{matrix}$.

Succinic acid crystallizes in colorless, quadrilateral needles, melting at 182°. It is fairly readily soluble in water, more difficultly in alcohol, and difficultly soluble in ether.

REACTIONS.

1. Heated in a hard-glass tube closed at one end, the acid melts and sublimes, being partly converted into succinic acid anhydride.

2. When heated on platinum-foil, it volatilizes, giving off vapors which have an extraordinarily strong power to provoke coughing.

3. If some neutral lead acetate is added to the aqueous solution, it remains clear at first, but if the solution is warmed gently and the mixture shaken, lead succinate separates as a heavy crystalline precipitate.

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QUANTITATIVE ANALYSIS.

I.

QUANTITATIVE ANALYSIS OF SOME INORGANIC COMPOUNDS.

1. DETERMINATION OF THE SULPHURIC ACID IN COPPER SULPHATE, $CuSO_4 + 5H_2O$.

Grind some perfectly pure copper sulphate in a mortar, dry it by pressing between filter-paper, weigh off exactly about 0.5 g. and place in a beaker (weigh a cork- or glass-stoppered weighing-tube approximately—to centigrams—introduce about 0.5 g., then weigh accurately, shake out the contents of the tube into a beaker and weigh again). Dissolve in 100 cc. of water, acidify with a few drops of hydrochloric acid, and heat on the wire gauze till it begins to boil. Heat to boiling about 10 cc. of barium chloride solution, acidified with a drop of hydrochloric acid, and cautiously add it to the copper sulphate solution. Heat the mixture on the water-bath until the barium sulphate has completely settled, filter through an ash-free, thin filter of 9 cm. diameter (Schleicher and Schüll's, No. 590, or Dreverhoff's "ash-free baryta-filter-paper," No. 400 or No. 412), and collect the precipitate without any loss on the filter with the aid of warm water and a glass rod, the end of which is covered with a piece

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of pure rubber tubing. Catch the filtrate, which must be perfectly clear, in a clean beaker, then wash the precipitate with hot water until the last wash-water is no longer made turbid by silver nitrate solution. Dry the filter with its contents (after it has been filled once with alcohol and once with ether), place it in a platinum or porcelain crucible, put the cover partly on and heat, at first gently and then more strongly, until the contents of the crucible appear pure white. Let cool in the desiccator, weigh, heat once more, cool, and weigh again. Heating with the blast-lamp is not advisable. Make two determinations. 233.46 parts by weight of barium sulphate correspond to 80 parts of SO_3. Copper sulphate contains 32.03 per cent. SO_3.

2. Determination of Copper in Copper Sulphate as Cupric Oxide.

Dissolve about 0.8 to 1 g. (accurately weighed) in 80 to 100 cc. of water in a porcelain dish, heat until it begins to boil, take away the flame and add, with constant stirring, dilute sodium hydroxide solution to alkaline reaction. Continue the heating on the water-bath or cautiously on the wire gauze until the precipitate has become entirely black, let settle, decant off the supernatant fluid through an ash-free filter, cover the copper oxide with water, heat again, let settle, decant again, etc., and repeat this operation once or twice more. Finally place the entire precipitate on the filter, wash thoroughly with hot water (the last wash-water must not become turbid when treated with hydrochloric acid and barium chloride solution), dry, and heat the precipitate, together with the filter, in an open porcelain crucible, at first gently and then strongly. In case some of the copper oxide sticks so firmly to the dish that it cannot be removed by the glass rod, dissolve it in a few drops of nitric acid, evaporate the solution on the water-bath in the porcelain crucible

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which is to be used for the determination, and heat the residue to red heat. Copper sulphate contains 31.87 per cent. of CuO.

3. Determination of Water of Crystallization in Copper Sulphate.

Place in a porcelain crucible a known weight—about 0.5 to 0.6 g.—of copper sulphate, weigh accurately, heat for some hours in an air-bath to 110–115°, and continue heating at this temperature until a constant weight is obtained. Copper sulphate loses four molecules of its water of crystallization at this temperature, i.e., 28.8 per cent.

4. Determination of Calcium in Calcium Carbonate, $CaCO_3$, as Calcium Oxide.

Place 0.3–0.4 g. (accurately weighed) of pure calcium carbonate, previously gently heated, in a 150- to 200-cc. beaker, cover with water, dissolve by cautiously adding dilute hydrochloric acid (the beaker should be covered with a watch-glass, which is afterwards to be rinsed off), and dilute with water, so that the beaker is at most one-third filled (or the carbonate may be dissolved in a flask, the solution poured into a beaker, and the flask well rinsed). Heat the solution in the beaker on the wire gauze until it begins to boil, and add about 15 cc. of ammonium oxalate solution. If a precipitate forms, it is dissolved by adding hydrochloric acid. Then, in any case, add ammonia to distinctly alkaline reaction, and after several hours of standing—best till next day—filter, wash the precipitate thoroughly with warm water (the last wash-water should give no turbidity with nitric acid and silver nitrate), dry, and incinerate. Finally heat strongly with the blast-lamp for at least five minutes. After weighing repeat the heating with the blast-lamp, and heat even a third time until the weighings agree. Test the calcium oxide obtained for calcium carbonate: when covered with

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water it must dissolve on the addition of hydrochloric acid without evolving any gas. Calcium carbonate contains 56 per cent. of calcium oxide.

5. Determination of Aluminium in Potash Alum, $AlK(SO_4)_2+12H_2O$, as Aluminium Oxide.

Dissolve about 1 g. of potash alum in 150 cc. of water in a porcelain dish, add 20 cc. of ammonium chloride solution, heat till boiling just begins, and add ammonia to faintly alkaline reaction (instead of ammonium chloride and ammonia, hydrochloric acid and ammonia may also be used). Heat for some time, until the ammonia is for the most part driven off, decant the supernatant liquid through an ash-free filter, wash a few times by decantation, bring the entire precipitate on the filter, wash with hot water until the wash-water no longer gives the reaction for chlorides, pour the filter full of alcohol, then full of ether. After these have evaporated, dry completely by heating in the air-bath and incinerate, at first very cautiously with the cover on the crucible, then heat for at least five minutes more with the blast-lamp, etc., etc. Potash alum contains 10.75 per cent. of Al_2O_3.

6. Determination of Chlorine in Sodium Chloride, NaCl.

Weigh accurately between 0.2 and 0.3 g. of pure sodium chloride (previously gently heated), place in a beaker, dissolve in about 100 cc. of water, acidify with a few drops of nitric acid, heat on the wire gauze, but not to boiling, then add silver nitrate solution as long as a precipitate forms. Continue the heating on the water-bath until the precipitate has well settled, decant off the fluid through an ash-free thin filter [1] of 9 cm. diameter, wash with hot water to which a few

[1] Use a Gooch crucible here and the method given on page 185.—O.

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drops of nitric acid have been added, and then bring the whole precipitate on the filter. Wash thoroughly with hot water (test the wash-water with hydrochloric acid), and dry the filter with its contents. Shake out the perfectly dry silver chloride as completely as possible on a piece of black glazed paper and cover it with a funnel. Place the folded filter in a weighed porcelain crucible, heat at first gently and then until the carbon is completely burned, let cool, drop on the ash in the crucible one to two drops of nitric acid (with a pipette), then one to two drops of hydrochloric acid, and evaporate the acids with extreme caution (water-bath or gently heated sand-bath, etc.). Heat somewhat more strongly, let cool a little and add the silver chloride from the glazed paper, carefully avoiding any loss (by using a brush or feather to remove the last traces of chloride). Heat the crucible gently till the chloride just begins to melt, let cool, and weigh. 143.38 parts of silver chloride correspond to 35.45 of chlorine. Sodium chloride contains 60.59 per cent. of chlorine.

In order to remove the silver chloride from the crucible fill it half-full of dilute sulphuric acid, put in a piece of zinc, and let stand till next day. The silver chloride is reduced to metallic silver, which may easily be removed from the crucible.

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II.

ANALYSIS OF THE URINE.

1. Determination of Urea according to Liebig.[1]

(*a*) Preparation of the Solution.

Dissolve 43 g. of yellow oxide of mercury (hydrargyrum oxydatum flavum via humida paratum, the red oxide cannot be used) in a mortar in a mixture of 100 cc. of nitric acid and the same amount of water. Place the solution together with the mercuric oxide, which still remains undissolved, in an evaporating-dish, heat on the water-bath, evaporate to a thin sirup, let cool, and dilute gradually with small portions of water to a volume of 550 cc., let stand till next day and filter through a dry filter.

(*b*) Standardizing the Solution.

For this purpose an exactly 2 per cent. solution of urea is required. Test the purity of the purest possible urea that can be obtained. The aqueous solution should not give any turbidity with nitric acid and silver nitrate solution, nor with hydrochloric acid and barium chloride solution; it should give no ammonia when boiled with a solution of sodium car-

[1] This method, which gives approximately the total amount of nitrogen in the urine, expressed as urea, is regarded by many as out of date. Moreover, it cannot be compared with the Kjeldahl method in accuracy. It is, however, simpler than the Kjeldahl determination and does not necessitate a complete laboratory, as the mercury solution can be bought and only needs to be tested. It is therefore still a valuable method.

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bonate. If the urea does not satisfy these tests it must be recrystallized from absolute alcohol.

Moreover, the urea must in any case be freed from the adhering hygroscopic water or tested as to its complete dryness. To do this, place about 2.5 g. of urea on a large watch-glass, weigh this accurately with the watch-glass, put into the desiccator, and weigh again after twenty-four hours. If the weight remains constant (a difference of 0.5 mg. may be neglected), the urea may be at once used; if not, put the watch-glass with the urea back into the desiccator and weigh again after twenty-four hours. Weigh off accurately on a previously weighed watch-glass exactly 2 g. of this urea, then shake it into a funnel placed in a 100-cc. measuring-flask, avoiding any loss, and rinse off the watch-glass carefully into the funnel with water from the wash-bottle. Wash the urea in the funnel into the flask with distilled water, rinse the funnel several times, dissolve by cautious shaking the urea which still remains in the flask, fill the flask up to the mark with distilled water, put in the stopper, and mix thoroughly.

When the urea solution is prepared, place the mercury solution in a burette (in case this is not dry it is washed out several times with small quantities of the mercury solution), and read the position of the fluid in the burette. Place 10 cc. of the urea solution in a small beaker, run in at first 17–18 cc. of the mercury solution in a continuous stream, and test the mixture to determine if it already contains an excess of mercury. For this purpose place a drop of the well-stirred mixture in a watch-glass filled with sodium carbonate solution. The watch-glass should rest upon black paper, and the drop should be allowed to flow in from the side. If a distinctly yellow color is perceptible alongside of the white mercury-urea compound, the end-point has been reached. If not, add about 0.3 to 0.5 cc. more of the mercury solution and test again, etc.

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The first determination is only approximate; it must be repeated several times. It is very important, as Pflüger has shown, to add at once, as nearly as possible, the number of cubic centimeters of the mercury solution which are necessary to complete the reaction. The mean of the several determinations is taken as correct. As a rule, the mercury solution is too strong and must be diluted. The amount of water necessary to be added (x) is calculated from the proportion

$$a : 20 - a = v : x;$$
$$x = \frac{v(20-a)}{a},$$

in which v is the volume of the mercury solution to be diluted and a the number of cubic centimeters used. Twenty cubic centimeters of the mercury solution will then correspond to 0.2 g. of urea.

(*c*) Determination of Urea in Urine.

As the urine always contains phosphoric acid and as phosphoric acid is also precipitated by the mercury solution, it is necessary in making a determination of urea in urine by this method to remove the phosphoric acid. This is done by mixing 50 cc. of the urine, accurately measured, with 25 cc. of Liebig's baryta mixture (2 volumes of baryta-water and 1 volume of barium nitrate solution) and filtering through a dry filter into a dry vessel. Measure off 15 cc. of the filtrate, which correspond to 10 cc. of the urine. The titration is performed in the same way as with the urea solution. In regard to the addition of the mercury solution, the specific gravity serves as a guide in the case of normal urines. The number of cubic centimeters of the mercury solution to be added at first is the same as the last two figures of the specific gravity (10 cc. if the specific grav-

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ity is 1.010). The number of cubic centimeters of the mercury solution used to obtain the end-point of the reaction expresses the amount of the urea in the urine in grams per liter. If considerably less than 30 cc. is found necessary to obtain the end-point, a correction (according to Liebig) must be applied. The difference between 30 and the number of cubic centimeters actually used is divided by 5. This number represents the tenths of cubic centimeters which must be subtracted from the number of cubic centimeters actually used. The entire determination is to be made at least twice on the same urine. It is also advisable to make a determination with a fever urine, in which case the detection of the end-reaction is more difficult, and the greater concentration of the urine may also cause difficulties (in the case of very concentrated urine take an equal quantity of urine and the baryta mixture; 15 cc. of the filtrate then correspond to 7.5 cc. of the urine; or the urine may previously be diluted). A determination of urea should also be made in a urine containing albumin, after removing the albumin.

Removal of Albumin from Urine.

One hundred cubic centimeters of urine are heated to boiling in a porcelain dish, the reaction being kept very faintly acid during the heating; if the urine is not acid, then add cautiously a few drops of acetic acid. The albumin then coagulates completely in large flakes. Boil gently for a few minutes, let cool, pour the fluid into a 100-cc. measuring-flask, carefully avoiding any loss, rinse out the dish with a small quantity of water, so that the volume will not exceed 100 cc., let cool completely (place in water), fill up to the mark with water, and filter through a dry filter.

Liebig's method gives approximately the total amount of nitrogen in the urine expressed as urea, but with an

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indeterminate error due to the presence of sodium chloride in the urine. This reacts with the mercuric nitrate to form mercuric chloride and sodium nitrate. It is customary, in order to diminish this error, to subtract a certain quantity from the amount of mercury solution used, 1 cc. for dilute, 1.5 cc. for concentrated urines (so-called correction for salt), but this correction is entirely arbitrary.

The above-described simple method, which is sufficient for the physician, has been very much improved by Pflüger; since, however, the description of Pflüger's method would require too much space, the reader is referred to the original work of Pflüger or to the larger text-books.

2. Direct Determination of Nitrogen in the Urine according to the Kjeldahl Method.

This method consists in the conversion of all the nitrogenous substances in the urine into ammonium sulphate, carbon dioxide, and water by heating them with concentrated sulphuric acid. Then add an excess of sodium hydroxide, distil off the ammonia into a receiver containing a definite quantity of an acid of known strength (standard acid), and determine by means of a standard solution of ammonia that part of the acid which has not been neutralized by the ammonia.

It is best to use a fifth-normal solution of hydrochloric acid and a tenth-normal solution of ammonia. These are prepared as follows:

Preparation of the Fifth-normal Solution of Hydrochloric Acid.

Dilute 19 cc. of pure concentrated hydrochloric acid, specific gravity 1.19, to 1100 cc. and mix thoroughly. To 25 cc. of this dilute acid add 2 cc. of strong nitric acid, specific gravity 1.2, and then a slight excess of a dilute solution of silver nitrate. An excess of silver nitrate may be shown to be present by allowing the precipitated silver chloride to

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settle and then adding a few drops more of the silver nitrate solution to the clear fluid, when no further precipitate of silver chloride will be formed. Heat to boiling with constant stirring, and continue the boiling for several minutes. Decant the clear liquid through a Gooch crucible, and wash the precipitate by decantation with 200 cc. of hot water containing 8 cc. of nitric acid (sp. gr. 1.2) and 2 cc. of a 1 per cent. silver nitrate solution. During the washing by decantation add the wash-water in small portions and break up the precipitate with a glass rod. Finally transfer the precipitate completely to the Gooch crucible, wash with 200 cc. of cold water and then with 25 to 30 cc. of 95 per cent. alcohol. Dry at 145–150° and weigh. Continue the heating at this temperature until the weight remains constant. From the weight of the silver chloride obtained calculate the equivalent in hydrochloric acid, as shown in the following example:

Twenty-five cubic centimeters of the dilute hydrochloric acid gave as the mean of two duplicate analyses 0.7751 g. AgCl. Molecular weight of AgCl = 143.38. AgCl : HCl : : 0.7751 : x. Molecular weight of HCl = 36.46. 143.38 : 36.46 : : 0.7751 : x;

$$x = 0.1971 \text{ g. HCl in 25 cc.}$$

$\frac{x}{25} = 0.007884$ g. HCl in 1 cc., or 7.884 g. HCl per liter. This may be converted into N/5 hydrochloric acid containing 7.292 g. ($\frac{1}{5}$ of 36.46) of HCl per liter, as follows:

$$7.292 : 1000 : : 7.884 : x;$$
$$x = 1081.1 \text{ cc.}$$

$x - 1000$ cc. = 81.1 cc., the amount of distilled water to be added to exactly one liter of the dilute hydrochloric acid (containing 7.884 g. HCl per liter) to make it fifth-normal hydrochloric acid (containing 7.292 g. HCl per liter).

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Preparation of the Tenth-normal Ammonia Solution.

Dilute 8 cc. of a concentrated solution of ammonia, specific gravity 0.9, to 1100 cc. Determine the strength of this diiute solution by titrating against 10 cc. portions of the standard N/5 hydrochloric acid, using a dilute solution of cochineal[1] as the indicator.

Example: 10 cc. of the standard N/5 HCl required 18.8 cc. of the NH_3 solution to exactly neutralize it (mean of two duplicate determinations).

$NH_3 + HCl = NH_4Cl$. 10 cc. N/5 HCl contain 0.07292 g. HCl.
17.07 36.46

HCl NH_3

$\therefore$ 36.46 : 17.07 : : 0.07292 : x;

$x = 0.03414$ g. NH_3 in 18.8 cc.

$\frac{x}{18.8} = 0.001816$ g. NH_3 in each cubic centimeter, or

1.816 g. of NH_3 per liter.

This may be made exactly a tenth-normal solution containing 1.707 g. of NH_3 per liter, as follows:

1.707 : 1000 : : 1.816 : x; $x = 1063.9$ cc.

$\therefore$ $x - 1000$ cc. $= 63.9$ cc.

Hence by adding 63.9 cc. of distilled water to exactly one liter of the dilute ammonia solution (found to contain 1.816 g. NH_3 per liter) the 1063.9 cc. of ammonia solution resulting will be exactly tenth-normal.

Ten cubic centimeters of the fifth-normal hydrochloric acid solution should be exactly neutralized by 20 cc. of the tenth-normal ammonia solution, using cochineal as the indicator.

[1] This solution of cochineal is prepared by digesting 3 grams of powdered cochineal in a mixture of 50 cc. of strong alcohol and 200 cc. of distilled water for a day or two at ordinary temperature. During the digestion the mixture should be frequently shaken. The filtered solution is employed as the indicator.

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Determination of the Total Nitrogen in Urine. (Kjeldahl Method.)

1. **Apparatus.** A Kjeldahl digestion-flask (250 cc.), an Erlenmeyer flask (1 liter), two accurate Schellbach burettes, a Reitmeyer bulb-tube, a Liebig's condenser, and the necessary stands.

2. **Reagents.** Pure concentrated sulphuric acid, specific gravity 1.84, an alcoholic solution (10 per cent.) of phenol phthaleïn, a strong solution of sodium hydroxide free from carbonates, pure crystallized copper sulphate ($CuSO_4 + 5H_2O$), pure crystallized potassium sulphate, a fifth-normal solution of hydrochloric acid, a tenth-normal solution of ammonia, a dilute solution of cochineal, and some granulated zinc.

3. **The Digestion.** Place 0.7 to 3.5 g. of the substance (5 cc. of urine or milk accurately measured from a burette), accurately weighed, in a 250-cc. Kjeldahl digestion-flask, add 20 cc. of pure concentrated sulphuric acid, specific gravity 1.84, and about half a gram of crystallized copper sulphate. Place the flask in an inclined position on a stand in the hood and heat over a low flame until frothing ceases and the blackened material begins to wash down the sides of the flask. Lower the flame and, when the contents of the flask have cooled somewhat, add from a 20-cc. test-tube 10 g. of crystallized potassium sulphate. Raise the heat gradually till the acid boils briskly, and continue to boil the solution till the acid becomes clear and has a pale-blue or green color. Then let cool.

4. **The Distillation.** When the contents of the digestion-flask are cold add *cautiously* about 100 cc. of distilled water and transfer the mixture by means of a 10-cm. funnel to a one-liter Erlenmeyer flask. Rinse the digestion-flask three or more times with 50 cc. portions of distilled water, so as to make the contents of the distillation-flask about 350–400 cc.,

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add a few drops of an alcoholic solution of phenol phthaleïn and a piece of granulated zinc. Connect the flask by means of a two-hole rubber stopper with a Reitmeyer bulb-tube attached to a Liebig condenser and with a bent safety-tube. Attach to the other end of the condenser a delivery-tube reaching to the bottom of a 250-cc. Erlenmeyer flask, which serves as a receiver. Place a carefully measured quantity of the N/5 hydrochloric acid (for 5 cc. of urine 25 cc. of the acid) in the receiver with a little of the cochineal solution and, if necessary, add a little water, so that the end of the delivery-tube dips below the surface of the liquid. Then add through the funnel-tube sufficient of the strong sodium hydroxide solution to make the contents of the distillation-flask distinctly alkaline. Mix the contents of the flask during this addition by means of a gentle rotary motion. Then distil off about 150 cc. of the liquid and titrate the contents of the receiver with the N/10 ammonia solution.

Example: 5 cc. of urine, specific gravity 1.027, were taken, 25 cc. of N/5 acid placed in the receiver required 6.2 cc. of the N/10 ammonia to completely neutralize after the distillation.

$$25 \text{ cc. of N/5 HCl} = 50 \text{ cc. of N/10 } NH_3.$$

$$\therefore 50 \text{ cc.} - 6.2 \text{ cc.} = 43.8 \text{ cc. } NH_3 \text{ obtained from the urine.}$$

$$\frac{43.8 \times 0.001404}{5 \times 1.027} = 1.197\% \text{ N in the urine.}$$

III. Determination of Uric Acid.

This determination is based on the precipitation of the uric acid in the presence of magnesium salts by an ammoniacal silver solution as silver magnesium urate, and the solubility of silver chloride in ammonia. To 200 cc. of the urine, whose specific gravity must not exceed 1020 (if it is more

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concentrated it must be correspondingly diluted), in a measuring-cylinder, add 50 cc. of magnesia mixture, to precipitate the phosphoric acid, then dilute with water to 300 cc. and filter at once through a dry filter into a dry vessel. Measure off 200 cc. of the filtrate and add 10 to 15 cc. of a 3 per cent. solution of silver nitrate. The precipitate must be flocculent and gelatinous; if it appears white, it contains too much silver chloride, then some ammonia must be added and the liquid well stirred. Single white spots of silver chloride in the precipitate do no harm; they are without influence on the accuracy of the uric acid determination. Let the precipitate settle, take out a small portion of the supernatant liquid with a pipette, place it in a test-tube, and acidify with nitric acid. The fluid must become cloudy from the formation of silver chloride—an indication that an excess of silver is present. If it does not do this, make the test-fluid alkaline again with ammonia, pour it back into the fluid containing the precipitate, and then add a few cubic centimeters more of the silver solution. Sometimes it is also necessary to add some more ammonia. Let the precipitate settle again and repeat the test. Now filter off the precipitate through an ordinary smooth filter (e.g., Schleicher and Schüll, No. 597), and carefully remove the precipitate sticking to the glass by means of a glass rod and water, so that none of it is lost. The precipitate is washed on the filter with water until a portion of the filtrate, when acidified with nitric acid, remains clear (absence of silver) and also shows only a very slight turbidity when silver nitrate is afterwards added (small amount of chlorides). Now place the funnel in a 400–500-cc. flask, pierce the filter, and carefully wash the precipitate into the flask and shake thoroughly. The volume of the mixture should amount to about 200–250 cc. Acidify with a few drops of hydrochloric acid, pass in hydrogen sulphide, shaking frequently, until the fluid is saturated, then

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heat just to boiling, filter, rinse out the flask with hot water, and wash the precipitate a few times with hot water.[1] The filtrate must be entirely clear and colorless. If it is quite dark-colored, it must at once be poured back on the filter before beginning the washing, and this must be repeated until it runs through clear or almost clear. If only a very little silver sulphide has passed through, this may be temporarily neglected. Evaporate the filtrate first over a free flame, then on the water-bath to a few cubic centimeters, add about five to eight drops of hydrochloric acid, and let stand till next day: the uric acid crystallizes and is usually only slightly colored.

If any silver sulphide separates during the evaporation, it may be filtered off. It is advisable, however, to do this at a time when the volume of the fluid has not been reduced more than one-half, as otherwise loss from the precipitation of the uric acid may occur. It is now necessary to determine the quantity of the uric acid. For this purpose dry a small filter in a watch-glass apparatus or in a weighing-tube (open) at 110–115°, and weigh the whole apparatus (closed). Bring the entire amount of the uric acid on the filter, using a part of the filtrate for the rinsing. When all the uric acid is on the filter, wash with a small quantity of water until a portion of the filtrate ceases to give any turbidity with nitric acid and silver nitrate solution. The filtrate and the wash-water are collected and measured. If possible, the volume of the filtrate and wash-water should not amount to more than 50–60 cc. Then wash twice with absolute alcohol and once with ether, place the filter in the watch-glass apparatus or in the weighing-tube, dry (open) and weigh (closed). The difference between the two weights is uric acid.

In calculating the results it is usual to apply a correction

[1] It is always advisable to examine the silver sulphide under the microscope to detect any uric acid that may be mixed with it.

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for the solubility of the uric acid; however, this is permissible only when the quantity of uric acid is not abnormally small and the quantity of the filtrate and wash-water does not exceed 60 cc. It is customary to add 0.5 mg. of uric acid for every 10 cc. of the filtrate plus the wash-water. The product of the amount obtained by 0.75 gives the percentage of uric acid in the urine.

The above-described method, though exact, is undeniably involved and somewhat difficult to carry out. The Hopkins method, in which the uric acid is precipitated as the ammonium salt and then titrated, gives apparently just as exact results. According to Folin [1] the following is the best procedure:

To 100 cc. of the urine (according to Wörner it is best to heat the urine previously to 40–45° C.) add 20 to 30 g. of powdered ammonium chloride or 30 g. of ammonium sulphate, and dissolve this by shaking. Filter off the precipitate after two hours and wash free from chlorine with a concentrated solution of ammonium sulphate and rinse into a flask with hot water. When cold add 15 cc. of concentrated sulphuric acid and titrate to a permanent rose-color with one-twentieth normal potassium permanganate solution (1.581 g. potassium permanganate to one liter; the solution is to be tested as to its correctness with one-twentieth normal oxalic acid or with iron ammonium sulphate). The temperature of the fluid titrated should be 60° to 63°. If it is higher, wait until it cools to this temperature. The number of cubic centimeters of potassium permanganate used multiplied by 3.75 [2] gives the quantity of the uric acid in milligrams.

Wörner [3] recommends the following procedure for the determination: 150 cc. of the urine are heated in a beaker

[1] Zeitschr. f. physiol. Chemie, 24, 224.
[2] Ibid.
[3] Ibid. 29, 70.

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to 40–50° and 30 g. of ammonium chloride dissolved therein. The precipitate of ammonium urate is filtered off after standing for one-half to one hour and washed free from chlorine with a 10 per cent. ammonium sulphate solution; then it is dissolved on the filter in hot 1 to 2 per cent. sodium hydroxide solution, the filter washed with hot water, and the filtrate and wash-water heated in a porcelain dish on the water-bath until all the ammonia has been driven off. The alkaline uric acid solution is rinsed into a Kjeldahl flask, stirred up with 15 cc. of concentrated sulphuric acid, some copper sulphate and potassium sulphate added, and the ammonia resulting estimated in the usual way.

One cubic centimeter of the fifth-normal hydrochloric acid corresponds to 8.4 mg. of uric acid. The heating of the alkaline uric acid solution may also be done in a large Kjeldahl flask, but then constant attention is necessary since loss may easily occur from foaming. In this case the entire determination may be carried to completion in the same flask.

IV. DETERMINATION OF CREATININE AS CREATININE ZINC CHLORIDE.

In this determination we proceed exactly as in the method given for the detection of creatinine, see page 102, using 80 cc. of the alcoholic filtrate for the precipitation with zinc chloride. The creatinine zinc chloride which separates is collected on a dried and weighed filter, as in the case of uric acid, washed with alcohol until the wash-liquid no longer gives the reaction for chlorides, dried and weighed. The amount obtained when multiplied by 0.39 gives the percentage of creatinine. (One hundred parts of creatinine zinc chloride correspond to 62.42 of creatinine, hence $\frac{0.6242 \times 5}{8}$ = in round numbers 0.39.)

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V. Determination of Ammonia.

Place in the crystallizing-dish of the Schlösing's apparatus 25 cc. of filtered urine and in the porcelain dish of the same apparatus 10 cc. of fifth-normal acid. Then add to the urine about 25 cc. of milk of lime (one part by weight of calcium hydroxide shaken with twelve parts of water) and quickly put on the cover. After forty-eight to seventy-two hours wash out the contents of the upper dish into a beaker, mix thoroughly, and titrate with tenth-normal ammonia solution. The difference corresponds to the ammonia evolved from the urine. One cubic centimeter of fifth-normal acid = 0.003414 g. NH_3. If 25 cc. of urine were used and fifth-normal acid, then the product of the difference in cubic centimeters by 0.013656 gives the quantity of ammonia in 100 cc. of urine. Test any water which has condensed on the top or walls of the apparatus for an alkaline reaction. If it reacts alkaline, rinse the apparatus with water and titrate it also.

VI. Determination of Urea.

(*a*) According to Mörner and Sjöqvist. Mix in a flask 5 cc. of urine, 5 cc. of baryta mixture (10 g. barium chloride, 3–4 g. barium hydroxide, and 100 cc. of water), and add 100 cc. of a mixture of alcohol and ether (two volumes of 97 per cent. alcohol and one volume of ether). Let stand till next day, filter, wash with the alcohol-ether mixture, evaporate at a gentle heat, and when the volume has reached 25 cc. add some water and some milk of magnesia (one part of burnt magnesia and twelve parts of water), and heat, to drive out ammonia, till the vapor no longer reacts alkaline. Then wash the fluid together with the precipitate into a Kjeldahl flask, add some dilute sulphuric acid, then 10 cc. of the concentrated acid, determine the nitrogen as usual

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and calculate it as urea. Not applicable to urines rich in hippuric acid.[1] The heating with magnesia may also be entirely omitted if the ammonia be determined separately and, calculated as urea, be deducted from the amount of urea found.

(*b*) According to Freund and Töpfer.[2] Five cubic centimeters of urine are treated with the same volume of alcohol and evaporated on the water-bath to dryness, the residue ground and extracted several times with absolute alcohol and filtered into a Kjeldahl flask. The alcohol is then driven off as completely as possible on the water-bath, about 70 cc. of a saturated ethereal solution of oxalic acid are poured into the flask, and the precipitate formed is allowed to settle. Filter the solution, leaving as much of the precipitate as possible in the flask, and wash with 60 to 80 cc. of ether in several portions.

When the ether has evaporated from the filter the contents of the filter are washed into the flask with distilled water and the solution of the contents of the flask is titrated with fifth-normal sodium hydroxide solution, using phenol phthaleïn (two drops of a 1 per cent. solution) as an indicator. Afterwards the determination of nitrogen according to Kjeldahl is undertaken.

VII. DETERMINATION OF OXALIC ACID.

The determination of oxalic acid is made according to the method given for the detection of this substance, page 104, but the extraction with ether must be made five times. Collect the calcium oxalate without loss on an ash-free filter, wash, dry, place in a weighed crucible, heat with the blast-lamp, to convert the calcium oxalate into calcium oxide

[1] Salaskin and Zaleski, Zeitschr. f. physiol. Chemie, 28, 73.
[2] Wiener klin. Rundschau, 1899, No. 23.

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(CaO), and weigh. This weight multiplied by 1.61 gives the quantity of the oxalic acid ($C_2H_2O_4$). After the weighing, dissolve the lime in a little dilute nitric acid: there should be no evolution of carbon dioxide. The solution obtained is tested with ammonium molybdate for phosphoric acid.

VIII. Determination of Phenol or Cresol.

Proceed as directed in the qualitative detection of these substances, page 108. Add bromine-water to the distillate until a permanent yellow color results, let stand a few days, filter through a weighed filter which has been dried in a desiccator over sulphuric acid, dry in the dark in the desiccator over sulphuric acid until the weight is approximately constant, and weigh. 331 parts of the precipitate correspond to 94 parts of phenol or 108 of cresol.

The object of letting the precipitate stand is for the purpose of converting the tetrabromcresol, $C_6H(CH_3)Br_3OBr$, which first forms into tribromphenol.[1]

IX. Determination of Albumin.

One hundred cubic centimeters or, in case of urines containing a large amount of albumin, only 50 cc. of the previously filtered and perfectly clear urine is placed in a beaker, which should be only half-filled by the liquid, a drop of acetic acid is added in case the reaction is not distinctly acid, and the mixture is heated for half an hour in the water-bath, so that the beaker is surrounded by the water, until the coagulum becomes coarsely flocculent. The water-bath should not be too hot in the beginning. If the albumin does not become coarsely flocculent, add a few more drops of acetic acid. Filter through a filter dried at 110–115°

[1] For the titration method of Kossler and Penny for determining phenol see C. Neuberg, Zeitschr. f. physiol. Chemie, 28, 123.

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and which is not too small, bring the albumin completely on the filter with the aid of a glass rod, wash with hot water until a portion of the wash-water no longer gives the reaction for chlorides, pour the filter full of absolute alcohol twice, then twice full of ether, dry at 110–115° to constant weight, and weigh. If the quantity of the albumin is considerable, it is necessary to incinerate the filter and albumin and to deduct the weight of the ash found from the weight of the albumin. In this case, of course, an ash-free filter must be used.

X. DETERMINATION OF GLUCOSE.

Two principal methods are in use, the determination by means of the optical rotation and the reduction of cupric oxide to cuprous oxide in alkaline solution. For practice in the determination of glucose, first use a 3 to 4 per cent. solution of glucose and then a diabetic urine or a 3 to 4 per cent. solution of glucose in urine.

(*a*) Determination by Means of Polarized Light.

Before using the polarization apparatus the correct position of the zero-point must be determined. All readings must be noted in figuring the mean. The filling of the tube is done as follows: First wash it out with distilled water, then two or three times with the solution to be analyzed. This precaution is absolutely essential. If it is omitted, streaks are formed in the fluid owing to the gradual mixing of the sugar solution with the water adhering to the walls of the tube. These interfere with the observation in exactly the same way as they would if in the glass cover-plate, or lens. After thorough rinsing, place the tube on the table, pour it full of sugar solution or urine, so that the fluid forms a rounded top, and slide on, from the side, the well-cleaned cover-plate, so that all air-bubbles are excluded. Then screw on the brass cap moderately tight. The cover must not

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be screwed on too tight, since the glass itself under very strong pressure may become optically active. With a new tube the cap is usually only lightly screwed on. A series of readings is always made and the numbers obtained noted; sometimes single observations fall entirely out of the series; these may be stricken out without hesitation.

The tube must be carefully cleaned immediately after using and also be rinsed with distilled water. This is especially important in the examination of urine. In putting the apparatus away the cover is only screwed on loosely in order that the rubber ring may not stick to the glass.

The urine must be perfectly clear and should always be filtered; further, it must not be too strongly colored. If it is not possible to get it perfectly clear by filtration or if it is too strongly colored, it must be treated with precipitating reagents, which also remove the coloring-matter. Neutral lead acetate is most generally used for this purpose. The urine is shaken with powdered lead acetate in a dry flask (to 50 cc. of the urine about 1 g. of the acetate) and filtered through a dry filter into a dry beaker. If the urine which passes through at first is turbid, pour it back repeatedly on the filter. Instead of this method of procedure we may also mix four volumes of urine with one volume of a saturated lead acetate solution and filter through a dry filter. The dilution must, of course, be taken into consideration in calculating the result. If the urine contains oxybutyric acid —which is always to be assumed when it contains acetoacetic acid—a correction must be applied to the number read for the amount of sugar, owing to the lævorotation caused by the oxybutyric acid. The urine is allowed to ferment, and then the rotation is determined. This is to be added to the rotation caused by the glucose. Combined glucuronic acid may also under certain circumstances cause lævorotation. (P. Mayer, Berl. klin. Wochenschr. 1900,

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No. 1.) The calculation of the amount of glucose is made as follows:

$$\frac{\alpha \times 100}{l \times 52.5} = \text{Number of grams of sugar in 100 cc. of urine,}$$

in which α = observed rotation using sodium light,

l = length of tube in decimeters.

(*b*) Determination by Means of Reduction.

Under certain definite conditions one molecule of glucose reduces very nearly five molecules or ten equivalents of copper oxide to cuprous oxide, hence 180 parts of anhydrous glucose will reduce the oxide from 1248.8 parts of crystallized copper sulphate, $CuSO_4 + 5H_2O$, to cuprous oxide.

1. FEHLING'S TITRATION METHOD.

Preparation of the Solutions. (*a*) 34.639 g. of pure copper sulphate in crystals, which have not effloresced, are accurately weighed off on a large watch-glass, dissolved by warming with water in a dish, the solution placed in a 500-cc. measuring-flask and, after it is perfectly cold, filled up to the mark.

(*b*) About 173 g. of potassium sodium tartrate (Rochelle salts) are dissolved by warming with a little water, the solution placed in a 500-cc. measuring-flask, 100 cc. of sodium hydroxide solution of 1.34 specific gravity added, and, after the mixture is cold, filled up to the volume of 500 cc.

Mix equal volumes of the two fluids—about 25 cc. measured with a pipette—in a dry beaker: deep-blue fluid, Fehling's solution, 10 cc. of which is equivalent to 0.05 g. of glucose. Test the solution by diluting a portion of it in a test-tube with about four times its volume of water and heating to boiling: no cuprous oxide should precipitate.

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The Determination.

Dilute the sugar solution or the urine so that the fluid contains about 0.5 per cent. of sugar or somewhat more, and put this solution into a burette.[1] Then measure off accurately with a pipette 10 cc. of the Fehling's solution into a moderately deep porcelain dish or into a flask, add about 40 cc. of water, heat to boiling, and then let the sugar solution run in. Red cuprous oxide or yellow cuprous hydroxide very soon precipitates. On further addition of the sugar solution the precipitate of the cuprous oxide increases, while the blue color of the solution decreases. It is now necessary to recognize the point when the blue color of the fluid disappears, i.e., when all the copper oxide has been reduced and yet no excess of sugar is present. When it is thought that this point is nearly reached, filter a small quantity of the fluid, removed by means of a pipette, through a small filter of very close filter-paper—the filtrate must contain no suspended cuprous oxide, which very readily goes through the filter—acidify with hydrochloric acid and make alkaline with ammonia: the fluid must not turn blue. If it does, then add 0.5 cc. more of the sugar solution, heat and test again for copper, etc. Of course this first titration is always only an approximation. If the first test shows that the solution is free from copper, it is possible that too much sugar solution has been added, and the entire determination must then be repeated and the sugar solution must be added more carefully. The strength of the dilute sugar solution in per cent. is equal to 5 divided by the number of cubic centimeters used to complete the reaction.

[1] The specific gravity is to be used to determine the amount of the dilution; of course it is not always possible at the first attempt to make the correct dilution.

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2. GRAVIMETRIC METHOD.

Thirty cubic centimeters of Fehling's solution are diluted with 50 cc. of water and heated to boiling in a porcelain dish. Twenty cubic centimeters of the diluted sugar solution are added, the solution is kept gently boiling for five minutes, and then it is diluted with about 120 cc. of water which has been previously boiled. The fluid must remain blue. Filter through a dried and weighed filter (Schleicher and Schüll, No. 590, about 9 cm., or the ash-free so-called baryta-filter-paper of Dreverhoff in Dresden), wash with hot water until a portion of the wash-water is no longer made turbid with hydrochloric acid and barium chloride, then with absolute alcohol and ether, dry at 110–115° and weigh. The difference in the two weights is the cuprous oxide. To calculate the amount of sugar from the cuprous oxide multiply by $\frac{18}{35.8} = 0.5028$.

More exact than the above method, but also more difficult to carry out, is the modification according to Allihn. In this the cuprous oxide is collected on an asbestos filter, reduced, by heating in a current of hydrogen, to metallic copper and weighed as such.

Since the reducing power of the sugar towards copper oxide is somewhat variable, according to the concentration of the sugar solution, it is not permissible, in very exact determinations, to calculate the amount of sugar from the quantity of copper obtained. In this case we must use an empirically determined table (pages 202, 203), which gives directly the quantity of sugar corresponding to the weight of the copper found.

Of course this table may also be used when the cuprous oxide itself has been weighed. It is only necessary to calculate this cuprous oxide as copper by multiplying by 318 and

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dividing by 358 (the equivalent weight of copper is 63.6; of cuprous oxide 71.6; and of cupric oxide 79.6). K. B. Lehmann [1] and von Riegler [2] have both given a method for the determination of sugar, which consists in heating the solution in which the sugar is to be estimated with a measured quantity of an excess of Fehling's solution, filtering from the precipitated cuprous oxide (or letting it settle), and determining by the iodometric method of de Haen the copper which remains in solution. The difference between the quantity of thiosulphate used and the amount of thiosulphate which the quantity of the Fehling's solution taken would require corresponds to the cupric oxide reduced by the sugar. This method has been tested and recommended by Benjamin.[3] The method of procedure as given by the two authors named is somewhat different. According to Lehmann, 60 cc. of the Fehling's solution is boiled with 25 cc. of the sugar solution, the mixture is then placed in a 250-cc. measuring-flask, and the flask filled up to the mark with water. Mix thoroughly, filter through a dry filter (or let settle), take out of the filtrate with a pipette 50 cc., make this slightly acid with sulphuric acid, add 2 to 3 g. of potassium iodide, about 3 cc. of starch paste (1 g. of arrowroot starch boiled with 100 cc. of water), and titrate with 1/10 normal thiosulphate solution the iodine set free:

$$2CuSO_4 + 4KI = 2K_2SO_4 + Cu_2I_2 + I_2,$$

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

Twenty cubic centimeters of Fehling's solution require 27.74 cc. of 1/10 normal thiosulphate solution. One cubic

[1] Arch. der Hygiene, 30, 267; Maly's Jahresber. f. Thierchemie, 1897, 64.

[2] Zeitschr. f. analyt. Chem. 37, 22.

[3] Deutsche med. Wochenschr. 1898, 551.

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TABLE TO DETERMINE THE AMOUNT OF GLUCOSE

Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.
10	6.1	43	22.4	76	38.8	109	55.5	142	72.3	175	89.5	208	106.8
11	6.6	44	22.9	77	39.3	110	56.0	143	72.9	176	90.0	209	107.4
12	7.1	45	23.4	78	39.8	111	56.5	144	73.4	177	90.5	210	107.9
13	7.6	46	23.9	79	40.3	112	57.0	145	73.9	178	91.1	211	108.4
14	8.1	47	24.4	80	40.8	113	57.5	146	74.4	179	91.6	212	109.0
15	8.6	48	24.9	81	41.3	114	58.0	147	74.9	180	92.1	213	109.5
16	9.0	49	25.4	82	41.8	115	58.6	148	75.5	181	92.6	214	110.0
17	9.5	50	25.9	83	42.3	116	59.1	149	76.0	182	93.1	215	110.6
18	10.0	51	26.4	84	42.8	117	59.6	150	76.5	183	93.7	216	111.1
19	10.5	52	26.9	85	43.4	118	60.1	151	77.0	184	94.2	217	111.6
20	11.0	53	27.4	86	43.9	119	60.6	152	77.5	185	94.7	218	112.1
21	11.5	54	27.9	87	44.4	120	61.1	153	78.1	186	95.2	219	112.7
22	12.0	55	28.4	88	44.9	121	61.6	154	78.6	187	95.7	220	113.2
23	12.5	56	28.8	89	45.4	122	62.1	155	79.1	188	96.3	221	113.7
24	13.0	57	29.3	90	45.9	123	62.6	156	79.6	189	96.8	222	114.3
25	13.5	58	29.8	91	46.4	124	63.1	157	80.1	190	97.3	223	114.8
26	14.0	59	30.3	92	46.9	125	63.7	158	80.7	191	97.8	224	115.3
27	14.5	60	30.8	93	47.4	126	64.2	159	81.2	192	98.4	225	115.9
28	15.0	61	31.3	94	47.9	127	64.7	160	81.7	193	98.9	226	116.4
29	15.5	62	31.8	95	48.4	128	65.2	161	82.2	194	99.4	227	116.9
30	16.0	63	32.3	96	48.9	129	65.7	162	82.7	195	100.0	228	117.4
31	16.5	64	32.8	97	49.4	130	66.2	163	83.3	196	100.5	229	118.0
32	17.0	65	33.3	98	49.9	131	66.7	164	83.8	197	101.0	230	118.5
33	17.5	66	33.8	99	50.4	132	67.2	165	84.3	198	101.5	231	119.0
34	18.0	67	34.3	100	50.9	133	67.7	166	84.8	199	102.0	232	119.6
35	18.5	68	34.8	101	51.4	134	68.2	167	85.3	200	102.6	233	120.1
36	18.9	69	35.3	102	51.9	135	68.8	168	85.9	201	103.1	234	120.7
37	19.4	70	35.8	103	52.4	136	69.3	169	86.4	202	103.7	235	121.2
38	19.9	71	36.3	104	52.9	137	69.8	170	86.9	203	104.2	236	121.7
39	20.4	72	36.8	105	53.5	138	70.3	171	87.4	204	104.7	237	122.3
40	20.9	73	37.3	106	54.0	139	70.8	172	87.9	205	105.3	238	122.8
41	21.4	74	37.9	107	54.5	140	71.3	173	88.5	206	105.8	239	123.4
42	21.9	75	38.3	108	55.0	141	71.8	174	89.0	207	106.3	240	123.9

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Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.	Copper. mg.	Glucose. mg.
241	124.4	273	141.7	305	159.3	337	177.0	369	195.1	401	213.5	433	232.2
242	125.0	274	142.2	306	159.8	338	177.6	370	195.7	402	214.1	434	232.8
243	125.5	275	142.8	307	160.4	339	178.1	371	196.3	403	214.6	435	233.4
244	126.0	276	143.3	308	160.9	340	178.7	372	196.8	404	215.2	436	233.9
245	126.6	277	143.9	309	161.5	341	179.3	373	197.4	405	215.8	437	234.5
246	127.1	278	144.4	310	162.0	342	179.8	374	198.0	406	216.4	438	235.1
247	127.6	279	145.0	311	162.6	343	180.4	375	198.6	407	217.0	439	235.7
248	128.1	280	145.5	312	163.1	344	180.9	376	199.1	408	217.5	440	236.3
249	128.7	281	146.1	313	163.7	345	181.5	377	199.7	409	218.1	441	236.9
250	129.2	282	146.6	314	164.2	346	182.1	378	200.3	410	218.7	442	237.5
251	129.7	283	147.2	315	164.8	347	182.6	379	200.8	411	219.3	443	238.1
252	130.3	284	147.7	316	165.3	348	183.2	380	201.4	412	219.9	444	238.7
253	130.8	285	148.3	317	165.9	349	183.7	381	202.0	413	220.4	445	239.3
254	131.4	286	148.8	318	166.4	350	184.3	382	202.5	414	221.0	446	239.8
255	131.9	287	149.4	319	167.0	351	184.9	383	203.1	415	221.6	447	240.4
256	132.4	288	149.9	320	167.5	352	185.4	384	203.7	416	222.2	448	241.0
257	133.0	289	150.5	321	168.1	353	186.0	385	204.3	417	222.8	449	241.6
258	133.5	290	151.0	322	168.6	354	186.6	386	204.8	418	223.3	450	242.2
259	134.1	291	151.6	323	169.2	355	187.2	387	205.4	419	223.9	451	242.8
260	134.6	292	152.1	324	169.7	356	187.7	388	206.0	420	224.5	452	243.4
261	135.1	293	152.7	325	170.3	357	188.3	389	206.5	421	225.1	453	244.0
262	135.7	294	153.2	326	170.9	358	188.9	390	207.1	422	225.7	454	244.6
263	136.2	295	153.8	327	171.4	359	189.4	391	207.7	423	226.3	455	245.2
264	136.8	296	154.3	328	172.0	360	190.0	392	208.3	424	226.9	456	245.7
265	137.3	297	154.9	329	172.5	361	190.6	393	208.8	425	227.5	457	246.3
266	137.8	298	155.4	330	173.1	362	191.1	394	209.4	426	228.0	458	246.9
267	138.4	299	156.0	331	173.7	363	191.7	395	210.0	427	228.6	459	247.5
268	138.9	300	156.5	332	174.2	364	192.3	396	210.6	428	229.2	460	248.1
269	139.5	301	157.1	333	174.8	365	192.9	397	211.2	429	229.8	461	248.7
270	140.0	302	157.6	334	175.3	366	193.4	398	211.7	430	230.4	462	249.3
271	140.6	303	158.2	335	175.9	367	194.0	399	212.3	431	231.0	463	249.9
272	141.1	304	158.7	336	176.5	368	194.6	400	212.9	432	231.6		

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centimeter of 1/10 normal thiosulphate solution corresponds to 6.36 mg. of copper.

If the quantity of cuprous oxide which separates is very small it may be well washed, dissolved in nitric acid, the nitrous acid removed by means of urea, and the copper directly determined. The results may easily be too high. (K. B. Lehmann.)

Very frequently in the case of urines containing a small amount of sugar the cuprous oxide does not precipitate. In this case neither the titration method nor the gravimetric method can be used. For such cases the following solution is recommended by Arthus: 125 cc. of Fehling's solution and 5 g. of ferrocyanide of potassium are diluted to one liter. Eight cubic centimeters of this solution correspond to 1 cc. of Fehling's solution. This solution is only decolorized by the glucose; however, the recognition of the end-point is rendered very uncertain by the color of the mixture.

Normal urine contains reducing substances corresponding to about 0.2 to 0.3 per cent. of glucose. If, therefore, we titrate a normal urine to which a known quantity of glucose has been added, the determination will of course give results correspondingly too high. For diabetic urine this error is apparently not so great and need not be considered, especially when the urine is diluted.

The determination of the sugar from the volume of the carbon dioxide evolved on fermenting the urine is very convenient, though less accurate. For this purpose we may use the empirically graduated "Fermentation Saccharimeter" of Einhorn, Fiebig, or Lohnstein, the latter also for undiluted urine (Allg. med. Centralzeitung, 1899, No. 101, and Münch. med. Wochenschr. 1899, No. 50).

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XI. Determination of Hydrochloric Acid.

Hydrochloric acid is usually expressed in the analyses as sodium chloride.

Mohr's Titration Method. Principle: If potassium chromate and then silver nitrate solutions be added to sodium chloride solution, silver chloride alone precipitates. Only after all the chlorine has combined with the silver does the silver chromate separate.[1] This mixes with the precipitated silver chloride and imparts to it an orange color.

The silver solution is advantageously prepared, so that 1 cc. corresponds to 0.01 g. of sodium chloride. This is obtained by dissolving 29.054 g. of pure fused silver nitrate in one liter of water or 7.2635 g. in 250 cc. (For the method of making up the solution see "Determination of Urea," page 181.)

Determination. To 10 cc. of the urine, in a porcelain dish or in a flask placed on white paper, add 100 cc. of water and then a few drops of a solution of potassium chromate until a distinct yellow color results. Then let the silver solution run in from a burette until the red color, which forms where the silver solution runs in, no longer disappears when the liquid is thoroughly stirred. The first trace of a permanent orange color marks the end-point of the reaction. The first titration gives only an approximate result. The determination is to be repeated once more with the same urine.

XII. Determination of the Total Sulphuric Acid.

Heat 100 cc.[2] of filtered, perfectly clear, urine in a beaker on the wire gauze to boiling with 10 cc. of hydrochloric acid. Keep boiling gently for about ten minutes, then remove the

[1] The silver phosphate precipitates after the chromate.

[2] In the case of concentrated urines 50 cc. + 50 cc. of water are sufficient.

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flame, and, after a few minutes, add cautiously 10–15 cc. of barium chloride solution which has been previously heated. Then let stand, preferably till next day, so that the barium sulphate may settle completely. If this does not take place, heat the beaker on the water-bath until the barium sulphate has settled and the fluid appears perfectly clear. Filter, after the heating on the water-bath, through a small, ash-free, close filter of 9 cm. diameter, and transfer the precipitate completely to the filter with the aid of a glass rod having a piece of rubber tubing on its end. The filtrate must be perfectly clear. If it is not, it is made so by pouring it repeatedly back on the filter. Test the clear filtrate by means of dilute sulphuric acid, to determine if sufficient barium chloride has been added, then wash the precipitate with warm water until a portion of the last wash-water is no longer rendered turbid with silver nitrate solution, pour the filter full of absolute alcohol once or twice to remove coloring matters (indigo blue and red) and to dry, and then once full of ether. To determine the quantity of the barium sulphate thus obtained place the filter, which is perfectly dry after some minutes, together with the precipitate, in a weighed platinum crucible, heat gently at first, with the cover partly on, and then more strongly for about five minutes or longer (with thick paper), at any rate until the contents of the crucible appear perfectly white, let cool and weigh. The difference in weight gives the quantity of barium sulphate. This weight multiplied by $\frac{98.08}{233.46} = 0.4201$ gives the quantity of sulphuric acid, and multiplied by $\frac{80.06}{233.46} = 0.34293$ the quantity of sulphuric anhydride.

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XIII. Determination of the Total Sulphur and of the Neutral Sulphur.

Fifty cubic centimeters of the urine, in the case of concentrated urines 25 cc., are evaporated in a platinum dish on the water-bath to a small volume, 20 g. of nitrate mixture (3 parts of saltpeter to 1 part of sodium carbonate) are added and heated cautiously from the side till the mass fuses completely and becomes white. On account of the sulphur present in illuminating-gas a spirit-flame is preferable for the heating. When cold dissolve the fused mass in water, rinse it into a flask, and cautiously run into the flask, very gradually through a funnel, 100 cc. of hydrochloric acid. Then heat on the sand-bath, with the funnel in the neck of the flask, until the evolution of gas has completely ceased, place in a porcelain dish, evaporate to dryness, pour on 100 cc. of hydrochloric acid, stirring thoroughly, again evaporate to dryness, and repeat this operation once more. Take up the dry residue with water, filter (to remove silicic acid) into a beaker, heat on the wire gauze till boiling begins, and add cautiously 10 cc. of hot barium chloride solution. Filter next day, etc., as in the determination of the total sulphuric acid.

By subtracting the amount of sulphur in the total sulphuric acid from the total sulphur we obtain the neutral sulphur.

XIV. Determination of Ethereal Sulphuric Acid.

For this determination it is best to use urine voided after the use of phenol or the urine of persons suffering with ileus.

Mix equal volumes (75 or 100 cc.) of urine and alkaline barium chloride solution (mixture of two volumes of baryta-water and one volume of barium chloride solution) in a dry beaker, stir thoroughly, and filter after a few minutes through

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a dry filter into a dry vessel. Measure off 100 cc. of the clear filtrate (on standing the filtrate becomes cloudy from the formation of barium carbonate), acidify very faintly with hydrochloric acid, then add 10 cc. more of hydrochloric acid and proceed as directed in the determination of the total sulphuric acid, only with the difference that the further addition of barium chloride solution is unnecessary.

XV. Determination of Phosphoric Acid.

Phosphoric acid is generally determined by titration with a solution of uranium acetate. If a solution of uranium (or uranyl) nitrate or acetate be added to a solution of secondary sodium phosphate acidified with acetic acid and containing sodium acetate, a yellowish-white precipitate of uranyl phosphate is formed according to the equation [1]

$$UO_2(NO_3)_2 + Na_2HPO_4 = UO_2HPO_4 + 2NaNO_3.$$

Any excess of uranium is readily detected: a drop of the mixture then gives, with a drop of potassium ferrocyanide solution, a brownish-red precipitate of uranyl ferrocyanide. This is the so-called end-reaction; it appears only when the phosphoric acid is completely precipitated and a slight excess of uranium is present. Instead of this some cochineal solution may be added to the solution of the phosphates: an excess of uranium produces a green color, but only when the fluid contains no free nitric acid. There is nothing to prevent using both end-reactions at the same time.

Preparation of the Uranium Solution.—Dissolve about 33 g. of commercial sodium uranate by warming with about 200 cc. of water and the smallest possible quantity of nitric

[1] As the urine contains monosodium phosphate the equation is

$$UO_2(NO_3)_2 + NaH_2PO_4 = UO_2HPO_4 + NaNO_3 + HNO_3.$$

The sodium acetate is added to get rid of the nitric acid set free in the reaction.

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acid, and dilute to 1100 cc. The strength of this solution must be empirically determined by titration with a solution of sodium phosphate of known strength. Weigh out accurately 10.0944 g. of pure, dry sodium phosphate, $Na_2HPO_4 + 12H_2O$, which has not effloresced, dissolve in water, and fill up to one liter. The preparation of this solution is, however, often very difficult, sometimes, indeed, even impossible, as the sodium phosphate effloresces in dry air. It is better, therefore, to proceed as follows: About 12 g. of sodium phosphate are dissolved in 1100 cc. of water; 50 cc. of the well-mixed solution are evaporated in a platinum or porcelain dish, dried and ignited. The residue, consisting of sodium pyrophosphate, $Na_4P_2O_7$, must weigh 0.1875 g. If it weighs more, the solution must be correspondingly diluted. Fifty cubic centimeters of this solution, measured off with a pipette, are run into a beaker, 5 cc. of acetic acid mixture (100 g. sodium acetate, 100 cc. of dilute acetic acid Ph. G. III diluted to one liter) added, then a few drops of cochineal tincture, and heated almost to boiling. Now run in about 18 cc. of the uranium solution and observe the color of the mixture. At the same time take out a drop of the solution with a glass rod and bring it into contact with a drop of potassium ferrocyanide solution (it is advisable to have a number of drops of the ferrocyanide arranged in series on a white porcelain plate). If a faint-brown color appears after a few moments, the end-reaction is attained (the mixture or the precipitate will then also show a greenish color). If the brown coloration does not appear, let more of the uranium solution run in and test each time, after the addition of 0.2 cc. When the end-point is reached heat the mixture for some minutes and test again for the end-reaction, etc. If the solution is of the correct strength, 50 cc. of the sodium phosphate solution will require 20 cc. of the uranium solution. Usually this is not the case, but less is required. If, for example, 19.4 cc. were used,

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then we must add to every 19.4 cc. 0.6 cc. of water. Twenty cubic centimeters of this uranium solution will then correspond to 0.1 g. P_2O_5.

The determination of the phosphoric acid in the urine is made exactly as described above in the titration and also with 50 cc. The number of cubic centimeters of the uranium solution used divided by 10 gives the amount of phosphoric acid, P_2O_5, in grams for one liter of urine.

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III.

ANALYSIS OF THE FÆCES.

The fæces, advantageously collected in a weighed dish (weighed with a glass rod for stirring), are dried by long-continued heating on a water-bath, stirring frequently with the glass rod, until they appear dry enough to powder. The drying may be facilitated, according to Poda,[1] by repeatedly pouring on absolute alcohol (after four to six hours add 50 cc. of absolute alcohol, then after another hour 25 cc., and heat again). Weigh the dish and determine thus the weight of the air-dried fæces. These are then quickly powdered and preserved in a well-closed, glass-stoppered bottle. The loss due to the removal of the contents of the dish and powdering makes no difference.

1. **Determination of the Amount of Water.** Weigh about 1.5 to 2 g. in a cork- or glass-stoppered weighing-tube (10 to 15 cm. long) whose weight is known to a centigram, shake out the contents of the tube into a weighed platinum dish, weigh the tube again, and thus determine the weight of the quantity used. Heat at 110° until the weight is constant. When required the amount of water may be calculated from the moist substance.

2. **The Determination of the Ash** is made with the same material. Heat the residue obtained in the determination of water cautiously until it ceases to give off vapor,

[1] Zeitschr. f. physiol. Chem. **25**, 355.

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then ignite completely by heating more strongly. If the complete oxidation to ash cannot be accomplished in this way, extract the residue on the water-bath with water, filter through a thin ashless filter, on which the ash containing carbon is also brought, so far as this may be done without difficulty. Rinse out the dish, and filter several times with hot water (of course rinsing water is used for washing the filter), and dry the filter with its contents. Dry the platinum dish also. Place the filter with the ash containing carbon in the platinum dish and ignite. The oxidation to ash now takes place quickly. Let cool, transfer the aqueous extract without loss to the platinum dish, evaporate to dryness, and ignite the residue. Naturally we may also evaporate the aqueous extract by itself. The soluble and the insoluble salts are thus separately determined.

3. **To Determine the Amount of Nitrogen** use 1 to 1.5 g. according to the amount of nitrogen present, which is greater with a meat diet than with mixed food. The exact determination of the weight of the fæces used is ascertained as in the case of the determination of the amount of water. The dust which remains sticking to the neck of the Kjeldahl flask is rinsed into the flask with a little water, 15 cc. of sulphuric acid and 0.5 g. of copper sulphate are added, and the flask is heated, at first with a small flame until the contents have a faint-blue or green color, etc. If the oxidation takes place slowly, it may be hastened by the cautious addition of a little finely pulverized permanganate. Thirty-five cubic centimeters of fifth-normal acid are placed in the receiver. This is sufficient for almost all cases. With fæces very rich in nitrogen use 20 cc. of half-normal acid (for the details of the process see under Urine, page 187).

4. **Determination of the Ether Extract (Fat).** Three or four grams of the material (accurately weighed) are extracted in a Soxhlet apparatus with ether, the ether extract

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evaporated in a light weighed Erlenmeyer flask, the last traces of ether driven out by means of a current of air or CO_2, and the flask heated for several hours at 80° or for a short time at 105° and weighed. If it is desired to know also the quantity of the fatty acids present in the form of soap, moisten a few grams of the material, accurately weighed, in a dish with dilute hydrochloric acid (1:3), dry on the water-bath, transfer the residue completely to the Soxhlet thimble, by wiping out the dish with pieces of filter-paper and placing these also in the thimble, and extract with ether. More ether extract will be obtained than in the first determination. The excess is due to the fatty acids present in the form of salts. If the residue has a brown color, it is better to extract it once more with ether. If the quantity of the fæces is small, the powder which has already been extracted with ether may be used for the determination of the fatty acids, or, if only a total determination of the fat and fatty acids is desired, the powder may be dampened with hydrochloric acid and dried on the water-bath before the extraction.

5. **Determination of the Starch or of the Carbohydrates according to Märker.** Place between 3 and 4 grams of the material (accurately weighed) in a porcelain vessel (the pots in which Liebig's extract of beef is packed are very suitable), pour on 25 cc. of a 1 per cent. solution of lactic acid and 30 cc. of water, stir thoroughly with a glass rod, rinse this off with the smallest possible quantity of water, and heat in an autoclave for two and one-half hours at three atmospheres pressure. By this means the starch will be converted into dextrin, while the cellulose will not be attacked. When there is no longer any pressure in the autoclave, it is opened, the contents of the porcelain vessel, together with the suspended matter, are placed in a 250-cc. measuring-flask, rinsing the vessel well with water and, when perfectly cold, the

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flask is filled up to the mark with water. Let settle and take out 200 cc. of the supernatant fluid (using a 100-cc. pipette); instead of this we may also filter through a dry filter and measure off 200 cc. of the filtrate. Place the measured fluid in a 400-cc. flask, add 15 cc. of hydrochloric acid, and heat two and one-half hours in an actively boiling water-bath, to convert the dextrin into glucose; let cool, place in a 300-cc. measuring-flask, nearly neutralize with caustic soda solution (if too much alkali has been added, make faintly acid again with dilute hydrochloric acid), and make the sugar determination—best gravimetrically with 50 cc.—or titrate according to Fehling (the latter method is to be recommended only with larger quantities of starch). Instead of this method, that of L. Liebermann, which requires no autoclave, may also be used. (See Analysis of Bread, page 229.)

6. **Determination of the Total Phosphorus.** (*a*) By fusing with the oxidizing mixture. About 1 to 1.3 g. of the material (accurately weighed) is fused with 20 g. of the oxidizing mixture. It is best to proceed as follows: About two-thirds of the oxidizing mixture is placed in a smooth mortar, a hole is made in the mixture with the pestle, the material placed in this, ground, and the mixture transferred to the platinum dish. The mortar is then rinsed out with the rest of the oxidizing mixture in two portions and, if necessary, wiped out with a feather or a brush. These portions are placed at the side of the dish, and the heating is also begun here and continued until all the organic substance is burned. It is very advantageous, towards the end of the operation, to hold the dish in the flame with the tongs. The complete combustion is then more readily accomplished. The fused mass is then dissolved in water, the solution introduced, without loss, by means of a funnel, into a flask, nitric acid cautiously added to strongly acid reaction (20 to 25 cc.) and heated on the sand-bath, with a funnel set in the neck of

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the flask, until the evolution of gas has entirely ceased. The fluid is then placed in a porcelain dish, evaporated to a volume of about 100 cc., 10 g. of ammonium nitrate and 50 cc. of the ammonium molybdate solution added, and let stand at room temperature or at 60° to 70° till next day. Decant through a small filter, and wash the dish and filter a few times with a solution containing 150 g. of ammonium nitrate and 10 cc. of nitric acid in the liter. Dissolve the yellow residue in the dish in dilute ammonia (1:3) by warming, filter the solution through the same filter, and wash dish and filter with ammonia. Now add to the solution contained in a beaker, whose volume should at most amount to 100 cc. (preferably less), hydrochloric acid until a yellow precipitate begins to separate again, then about one-fourth of the volume of ammonia and 10 cc. of magnesium chloride mixture. Filter next day, transfer the precipitate of $MgNH_4PO_4$ with the help of the filtrate completely to the filter, then wash with dilute ammonia (1:3) until a portion of the filtrate when acidified with nitric acid is no longer rendered turbid with silver nitrate solution or only faintly, dry, and ignite strongly. The magnesium pyrophosphate obtained must be white, or at most a very faint gray. If it is not possible to attain this by ignition alone, add a little nitric acid, dry, and ignite again. 111.36 parts of $Mg_2P_2O_7$ correspond to 31 parts of phosphorus = 71 parts of P_2O_5.

(*b*) Instead of fusing with the oxidizing mixture the organic matter may also be destroyed, according to A. Neumann,[1] by heating with sulphuric acid and ammonium nitrate. To 2 to 3 g. of the material take 15 cc. of sulphuric acid and 15 g. of ammonium nitrate. As in the nitrogen determination, the powdered fæces are placed in a Kjeldahl flask, washed down with a little water, 15 cc. of sulphuric

[1] Chem. Centralbl., 1898, **1**, 219.

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acid added, and heated till colorless. The ammonium nitrate is added in three portions after cooling each time. When cold, make alkaline with ammonia and acidify with acetic acid. In case no precipitate (of ferric phosphate) is formed, the phosphoric acid may be titrated with the uranium solution; otherwise it must be determined gravimetrically. We may also in any case acidify with nitric acid and precipitate with ammonium molybdate solution.

Pfeiffer and Scholz (Deutsches Arch. f. klin. Med. **63**, 373) recommend to heat 0.5 to 1.0 g. of the dried fæces with 10 cc. of sulphuric acid and 5 g. of potassium sulphate and to precipitate the diluted solution with ammonia and magnesia mixture. The precipitate is filtered off, dissolved in dilute acetic acid, together with the ammonium magnesium phosphate clinging to the beaker (the volume of the solution should not exceed 50 cc.); the solution is then neutralized with caustic soda, 5 cc. of acetic acid mixture added (see under Urine), and titrated with the uranium solution (the method does not take into consideration the ferric phosphate, which is seldom entirely absent; the analyses given, however, show concordant results).

7. Determination of the Total Sulphur. Fuse about 2 g. with 30 g. of the oxidizing mixture, then proceed as directed in the determination of sulphur in the urine, page 207.

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IV.

ANALYSIS OF MEAT.

In all determinations finely chopped meat is used. Great care should be taken that the material used for the single determinations should represent as far as possible a correct average sample.

1. **Determination of the Amount of Water.** Between 2 and 3 g., accurately weighed, are placed in a platinum (or porcelain) dish and dried to constant weight, at first on the water-bath, then at 110° to 115°.

2. **Determination of the Amount of Ash.** The same sample serves for the determination of the amount of ash. Cautiously char the substance at first, then heat until vapors no longer escape, grind the carbon with the agate pestle or a glass rod, extract with hot water, filter through an ash-free filter, wash thoroughly, and preserve the filtrate. Now dry the filter with the carbon, put it in the dish, and ignite completely. When the dish is cold add the filtrate, evaporate to dryness, dry and ignite. See "Analysis of the Fæces," page 211.

3. **Determination of the Amount of Nitrogen.** The amount of nitrogen may be determined directly in fresh meat, but the operation is not very easy and the determination after drying is to be preferred. We proceed best as follows: A considerable quantity, about 50 g., of meat is accurately weighed in a dish together with a glass rod, and dried on the water-bath until the meat may be powdered. Now

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weigh the dish with its contents, remove the dry meat completely with a spatula, and proceed as directed in the method for the "Determination of Nitrogen in the Fæces," using 0.5 g. of the material and 35 cc. of the fifth-normal acid. From the value obtained the percentage of nitrogen in the fresh meat is calculated. From this the amount in the dried meat may be estimated by making use of the determination of the amount of water in the fresh meat (see 1 above).

There is no reason why the determination of the amounts of water and ash may not be made with the half-dried meat instead of with the fresh. Where extreme accuracy is required the meat is to be dried in a vacuum over sulphuric acid instead of on the water-bath (Pflüger and Argutinsky). If it is desired to make the nitrogen determination with the fresh meat, weigh it off on a piece of tin-foil, fold this, place it in the Kjeldahl flask, and determine the nitrogen in the usual manner.

4. **Determination of Fat or Determination of the Ether Extract.** This is best made with the fresh meat. According to the amount of fat present, weigh off accurately an average sample of 3 to 5 g. in a large weighing-glass, pour over it about 30 cc. of absolute alcohol, stir thoroughly with a thin glass rod, which is then washed off with alcohol, cork, and let stand for twenty-four hours. Filter and transfer the meat powder completely to the filter. Evaporate the alcoholic extract to dryness and treat the residue with ether, filter, wash with ether, and place the ether extract, after concentrating, in the Soxhlet extraction-flask. Place the filter containing the meat powder in the thimble of the Soxhlet apparatus. For further details see "Determination of Fat in the Fæces," page 212.

Pflüger and Dormeyer recommend, instead of this method, one which depends on the dissolving of the meat by digestion and extraction of the solution obtained with ether (Pflüger's

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Archiv, **65**, 90, 1896). This method gives somewhat higher results, however, and there is more chance of lactic acid being mixed with the fat (or fat + cholesterin + lecithin) in this method than in the one above described.

5. **Determination of the Total Phosphorus.** This determination is made in the same way as the corresponding determination with the fæces, 1–1.5 g. of the meat powder being fused with 20 to 30 g. of the oxidizing mixture (using 20 to 25 or 30 to 35 cc. of nitric acid).

6. **Determination of the Total Sulphur.** This determination may be made with the fresh meat as well as with the dried powder.

(*a*) **Method with the Fresh Meat.** About 5 g. of meat are accurately weighed, placed in a long-necked flask (the residue sticking to the vessel is washed in with nitric acid), covered with nitric acid of about 1.48 specific gravity, and heated with this on the water-bath until the development of red fumes has completely ceased. Dilute the solution with water, place it in a porcelain dish (if the quantity of fat is very large, the solution must be filtered when perfectly cold and the filter thoroughly washed), evaporate on the water-bath to dryness, dissolve the residue in 5 to 6 g. of dry sodium carbonate (which must be absolutely free from sulphates) and water, place in a platinum dish, add 3 g. of potassium nitrate, evaporate to dryness, and heat slowly till fused. When cold, dissolve the perfectly white fused mass in water, heat the solution in a flask (in the neck of which a funnel is placed) with hydrochloric acid until red fumes cease to escape, and evaporate in a porcelain dish on the water-bath to complete dryness. Then evaporate twice more with hydrochloric acid, dissolve in water (if the solution is not clear it must be filtered from the silicic acid and the filter thoroughly washed), precipitate the hot solution with barium chloride, and filter after twenty-four hours, etc.

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233.46 parts $BaSO_4$ = 32.06 parts sulphur. See the "Determination of Sulphur in Urine," page 207.

(*b*) With the dry meat powder the determination is made with about 1.5 g. and 30 g. of the oxidizing mixture in the same way as in the "Determination of Sulphur in the Urine," see page 207.

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V.

ANALYSIS OF MILK.

1. Determination of the Amount of Water. Place 5 or 10 cc. of milk in a weighed dish, preferably a platinum dish, evaporate to dryness on the water-bath, heat to constant weight at 105°, and weigh.

If the greatest possible accuracy is desired, the milk must be weighed, not measured, and the dry residue must also be protected from the air in order that it may not take up water during the weighing. This applies to all similar determinations. Both these objects may be attained by using a platinum crucible for the determination. This is placed in a large weighing-glass, the glass is then closed and the weight of the whole determined. Five to ten cubic centimeters of the milk are then introduced into the crucible, the weighing-glass is closed, and the quantity of the milk is determined by weighing again. The crucible containing the dry residue is also weighed in the weighing-glass.

2. Determination of the Amount of Ash. The dry residue is carefully carbonized, then more strongly heated, but not ignited at too high a heat, until the carbon is completely burned. If complete combustion cannot be attained in this way, extract the half-burned residue by cautiously warming with water, filter through an ashless filter, etc., as described under "Fæces" and "Meat."

3. Determination of Fat. (*a*) Let 5 to 10 cc. of milk drop on kaolin or burnt gypsum or sand, contained in the

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paper cartridge of the Soxhlet extraction apparatus (in case a closed Schleicher and Schüll extraction-thimble is not available, this cartridge may be placed in a cylinder made out of perforated sheet metal and closed at one end), dry by long heating at 105°, and extract for three hours in the Soxhlet apparatus with anhydrous ether.

The determination of the dry residue may also be combined with the determination of the fat. For this purpose dry the extraction-thimble containing the kaolin and the milk to constant weight. Determine the loss of weight which it undergoes when extracted with ether. This must be the same as the weight of the fat. Frequently traces of kaolin or gypsum are carried over mechanically into the ether extract.[1] In this case the ether extract must of course be filtered before it is evaporated, and then, too, the weight of the fat will not coincide with the loss of weight of the extraction-thimble.

(*b*) Warm 25 cc. of milk with the same amount of hydrochloric acid of 1.12 specific gravity for some time in a flask on the water-bath, let cool, and transfer to a separating-funnel, rinsing with warm water. Rinse the flask several times with ether, which is poured into the separating-funnel until the volume of the ether equals that of the aqueous fluid. Then shake with the ether, separate the ether extract, and shake once or twice more with ether. The ether extracts are freed from hydrochloric acid by shaking with water and filtered through a dry filter which is washed with ether. By evaporating the ether the fat is obtained. This frequently requires to be purified by dissolving once more in ether.

4. **Determination of the Total Nitrogen according to Kjeldahl.** Five cubic centimeters of milk are heated in a Kjeldahl flask with 20 cc. of concentrated sulphuric acid,

[1] When a paper cartridge made by Schleicher and Schüll is used this is less liable to occur.

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0.5 g. of copper sulphate, and 10 g. of potassium sulphate, at first gently and then more strongly, until the contents of the flask have a light-blue or green color. Place 25 cc. of fifth-normal acid in the receiver. For further details see the "Determination of Nitrogen according to Kjeldahl" in the chapter on "Urine."

If the amount of proteid be calculated by multiplying the amount of nitrogen obtained by 6.25, as is customary, the result will be somewhat too high. According to I. Munk, it is better to use the factor 6.0 for cow's milk, and for human milk 5.77. See the "Determination of Total Proteid" below.

5. **Separate Determination of Casein and Albumin (according to Schlossmann[1]).** Dilute 10 cc. of milk with 3–5 parts of water and cautiously warm to 40° over a free flame or, better, in a water-bath, then add 1 cc. of concentrated solution of potash-alum and stir thoroughly. If a flocculent precipitate, which settles quickly, does not form, then continue to add the alum solution, 0.5 cc. at a time, until the coagulation and precipitation take place completely. Of course time (half a minute) must be allowed before each addition of the alum solution for the settling of the precipitate; the temperature is to be kept constant at 40°. A slight excess of the alum solution makes no difference. After the completion of the precipitation let stand a few minutes and then filter. When the filtrate is perfectly clear, which may require filtering two or three times through the same filter, wash the precipitate a few times on the filter with water and determine the nitrogen by the Kjeldahl method. From the amount of nitrogen found calculate the amount of casein by multiplying by 6.37.

Add to the filtrate 10 cc. of tannin solution,[2] filter off the

[1] Zeit. f. physiol. Chemie, 22, 213.

[2] The mixture recommended by Almen, consisting of 4 g. of tannin,

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voluminous precipitate which forms, and, after washing three times with fresh water, determine the amount of nitrogen by the Kjeldahl method. From the amount of nitrogen found calculate the amount of albumin and globulin by multiplying by 6.37.

6. Determination of the Total Proteid according to Ritthausen and I. Munk.[1] Place 10 cc. of human or cow's milk in a 250-cc. beaker, dilute with water to 100 cc. (with human milk dilution to 60 cc. is sufficient), heat, and add 1 to 2 cc. of alum solution, then when the fluid just begins to boil 2 to 5 cc. of a paste of cupric hydroxide, and continue the boiling for some minutes. The finely flocculent precipitate, which settles quickly, as soon as the mixture has been coagulated by heating, is filtered while still warm, washed on the filter with hot water, and the whole filter treated while still moist according to Kjeldahl.

The cupric hydroxide is prepared according to Stutzer as follows: 100 g. of crystallized copper sulphate are dissolved in 5 liters of water and 2.5 g. of glycerine are added. Dilute sodium hydroxide solution is then added until the fluid reacts alkaline, the cupric hydroxide is then filtered off and ground with water containing 5 g. of glycerine to the liter. By repeated decanting and filtering the last traces of alkali are removed. The product remaining on the filter is then ground and diluted with water which contains 10 per cent. of glycerin, so that it forms a homogeneous mass which may be measured out with a pipette. This is kept in the dark in a well-closed bottle. The amount of copper oxide in the pasty mass may be determined by evaporating a measured volume

8 cc. of 25 per cent. acetic acid, and 190 cc. of 40 to 50 per cent. alcohol, gives the best results.

[1] See Ritthausen, Jour. f. prakt. Chemie N. F. 15, 329; Emil Pfeiffer, Analyse der Milch. Wiesbaden, 1887. I. Munk, Virchow's Arch. 134, 501 (1893).

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to dryness and igniting the residue. It is advisable only to prepare small quantities at a time, using about 20 g. of copper sulphate.

7. **Determination of Milk-sugar.** Dilute the filtrate and wash-water freed from the albumin (see the "Determination of Total Proteid according to Ritthausen and Munk" or the method of Soxhlet given below) to a definite volume (with 20 cc. of milk to 140 or 160 cc.), fill a burette with this solution, and titrate 20 cc. of Fehling's solution + 80 cc. of water with it. (See chapter on "Urine.") Twenty cubic centimeters of Fehling's solution correspond to 0.135 g. of anhydrous milk-sugar ($C_{12}H_{22}O_{11}$).

Instead of titrating we may add to 40 cc. of Fehling's solution + 80 cc. of water, heated to boiling, 30 cc. of the above fluid, continue the heating for six to seven minutes, collect the precipitated cuprous oxide on a weighed filter and weigh as such, or convert it into copper sulphide or metallic copper and weigh this. We may also remove the casein and albumin in one operation according to Soxhlet. Twenty-five cubic centimeters of milk are mixed with 400 cc. of water, a few drops of acetic acid are added, and the solution heated to boiling. When cold dilute to 500 cc. and filter through a dry filter. One hundred cubic centimeters of the filtrate = 5 cc. of milk are then boiled with 50 cc. of Fehling's solution for six minutes, etc. Since the reduction equivalent of milk-sugar for copper oxide in alkaline solution is not constant, according to Soxhlet, but varies according to the concentration of the milk-sugar solution, an empirical table (see pages 226 and 227) must be used for the calculation. This has been established by Soxhlet and calculated directly for milk-sugar by E. Wein.

8. **Determination of the Total Phosphorus.** Ten cubic centimeters of milk are dropped upon 30 g. of the oxidizing mixture, contained in a platinum dish, evaporated to dry-

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TABLE TO DETERMINE THE AMOUNT OF

Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.
100	71.6	**135**	97.6	**170**	123.9	**205**	150.7
101	72.4	**136**	98.3	**171**	124.7	**206**	151.5
102	73.1	**137**	99.1	**172**	125.5	**207**	152.2
103	73.8	**138**	99.8	**173**	126.2	**208**	153.0
104	74.6	**139**	100.6	**174**	127.0	**209**	153.7
105	75.3	**140**	101.3	**175**	127.8	**210**	154.5
106	76.1	**141**	102.0	**176**	128.5	**211**	155.2
107	76.8	**142**	102.8	**177**	129.3	**212**	156.0
108	77.6	**143**	103.5	**178**	130.1	**213**	156.7
109	78.3	**144**	104.3	**179**	130.8	**214**	157.5
110	79.0	**145**	105.1	**180**	131.6	**215**	158.2
111	79.8	**146**	105.8	**181**	132.4	**216**	159.0
112	80.5	**147**	106.6	**182**	133.1	**217**	159.7
113	81.3	**148**	107.3	**183**	133.9	**218**	160.4
114	82.0	**149**	108.1	**184**	134.7	**219**	161.2
115	82.7	**150**	108.8	**185**	135.4	**220**	161.9
116	83.5	**151**	109.6	**186**	136.2	**221**	162.7
117	84.2	**152**	110.3	**187**	137.0	**222**	163.4
118	85.0	**153**	111.1	**188**	137.7	**223**	164.2
119	85.7	**154**	111.9	**189**	138.5	**224**	164.9
120	86.4	**155**	112.6	**190**	139.3	**225**	165.7
121	87.2	**156**	113.4	**191**	140.0	**226**	166.4
122	87.9	**157**	114.1	**192**	140.8	**227**	167.2
123	88.7	**158**	114.9	**193**	141.6	**228**	167.9
124	89.4	**159**	115.6	**194**	142.3	**229**	168.6
125	90.1	**160**	116.4	**195**	143.1	**230**	179.4
126	90.9	**161**	117.1	**196**	143.9	**231**	170.1
127	91.6	**162**	117.9	**197**	144.6	**232**	170.9
128	92.4	**163**	118.6	**198**	145.4	**233**	171.6
129	93.1	**164**	119.4	**199**	146.2	**234**	172.4
130	93.8	**165**	120.2	**200**	146.9	**235**	173.1
131	94.6	**166**	120.9	**201**	147.7	**236**	173.9
132	95.3	**167**	121.7	**202**	148.5	**237**	174.6
133	96.1	**168**	122.4	**203**	149.2	**238**	175.4
134	96.9	**169**	123.2	**204**	150.0	**239**	176.2

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MILK-SUGAR FROM THE WEIGHT OF COPPER.

Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.	Copper. mg.	Milk-sugar. mg.
240	176.9	**275**	204.3	**310**	232.2	**345**	259.8
241	177.7	**276**	205.1	**311**	232.9	**346**	260.6
242	178.5	**277**	205.9	**312**	233.7	**347**	261.4
243	179.3	**278**	206.7	**313**	234.5	**348**	262.3
244	180.1	**279**	207.5	**314**	235.3	**349**	263.1
245	180.8	**280**	208.3	**315**	236.1	**350**	263.9
246	181.6	**281**	209.1	**316**	236.8	**351**	264.7
247	182.4	**282**	209.9	**317**	237.6	**352**	265.5
248	183.2	**283**	210.7	**318**	238.4	**353**	266.3
249	184.0	**284**	211.5	**319**	239.2	**354**	267.2
250	184.8	**285**	212.3	**320**	240.0	**355**	268.0
251	185.5	**286**	213.1	**321**	240.7	**356**	268.8
252	186.3	**287**	213.9	**322**	241.5	**357**	269.6
253	187.1	**288**	214.7	**323**	242.3	**358**	270.4
254	187.9	**289**	215.5	**324**	243.1	**359**	271.2
255	188.7	**290**	216.3	**325**	243.9	**360**	272.1
256	189.4	**291**	217.1	**326**	244.6	**361**	272.9
257	190.2	**292**	217.9	**327**	245.4	**362**	273.7
258	191.0	**293**	218.7	**328**	246.2	**363**	274.5
259	191.8	**294**	219.5	**329**	247.0	**364**	275.3
260	192.5	**295**	220.3	**330**	247.7	**365**	276.2
261	193.3	**296**	221.1	**331**	248.5	**366**	277.1
262	191.4	**297**	221.9	**332**	429.2	**367**	277.9
263	194.9	**298**	222.7	**333**	250.0	**368**	278.8
264	195.7	**299**	223.5	**334**	250.8	**369**	279.6
265	196.4	**300**	224.4	**335**	251.6	**370**	280.5
266	197.2	**301**	225.2	**336**	252.5	**371**	281.4
267	198.0	**302**	225.9	**337**	253.3	**372**	282.2
268	198.8	**303**	226.7	**338**	254.1	**373**	283.1
269	199.5	**304**	227.5	**339**	254.9	**374**	283.9
270	200.3	**305**	228.3	**340**	255.7	**375**	284.8
271	201.1	**306**	229.1	**341**	256.5	**376**	285.7
272	201.9	**307**	229.8	**342**	257.4	**377**	286.5
273	202.7	**308**	230.6	**343**	258.2	**378**	287.4
274	203.5	**309**	231.4	**344**	259.0	**379**	288.2

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ness, and fused.[1] Then proceed as directed under "Fæces," page 214, using 30 to 35 cc. of nitric acid. Instead of this method we may also use sulphuric acid and ammonium nitrate for the oxidation according to the method of A. Neumann (see page 215).

9. **Determination of the Total Sulphur.** Ten cubic centimeters of milk are evaporated to dryness with 30 g. of the oxidizing mixture and fused. Then proceed as directed under "Urine," page 207.

[1] By working very carefully the oxidising mixture may be heated to fusion directly after adding the milk.

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VI.

ANALYSIS OF BREAD, ETC.

For the analysis of white bread it is best to take a whole roll, weigh it, cut it up over a sheet of paper into slices about a centimeter thick, place them without loss in an evaporating-dish, weigh again as a check, heat on the water-bath or in an air-bath until the pieces are dry enough to be powdered, let cool, weigh, grind, and place the powder in a tight glass-stoppered bottle. This material is used for the analysis. If the volume of a single roll is too large for this method of procedure, a mixture of the crust and crumb is prepared, which corresponds as nearly as possible to the proportion of crust and crumb of the bread, and with this we proceed as directed above.

For the determination of the amount of water and ash take 2 to 3 g. of the powder; for the nitrogen determination, about 1.5 to 1.8 g., placing 25 to 30 cc. of fifth-normal acid in the receiver (the digestion must be conducted very carefully at first); for the determination of fat, extract about 4 g. in a Soxhlet apparatus, or, better, boil the same quantity with 75 to 100 cc. of dilute hydrochloric acid (1:2) till dissolved and then extract with ether; the dry residue obtained by evaporating the ether extract must be again dissolved in ether to purify it. For the determination of the carbohydrates, heat 2 to 3 g. (of course all these quantities are to be accurately weighed), according to the method of Märker, with lactic acid solution, etc. (See the "Determination of

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the Carbohydrates in the Fæces," page 213.) If the sugar is to be determined gravimetrically, take 50 cc. of the Fehling's solution, 100 cc. of water, and 25 cc. of the solution ultimately obtained from the bread. For further details of the methods consult the earlier chapters.

According to L. Liebermann [1] this method gives too low results on account of the destruction of sugar by the long-continued heating. He recommends the following method: About 10 g. of the substance are boiled for one and one-half hours on a sand-bath with 100 cc. of 2 per cent. hydrochloric acid in a 250–300-cc. flask connected with a reflux condenser. Then the fluid is almost neutralized with sodium hydroxide solution, filtered into a liter measuring-flask, rinsing and washing with hot water. Dilute to one liter and take out 20 cc. for the sugar determination with Fehling's solution. According to L. Liebermann there is no danger of the 2 per cent. hydrochloric acid converting the cellulose into sugar. Instead of filtering and washing, the fluid, together with suspended material, may be diluted to one liter and then filtered through a dry filter.

Liebermann uses a peculiar method for the determination of the cuprous oxide. The precipitated oxide is filtered off, washed, dissolved in hydrochloric acid, and the solution reduced in a weighed platinum dish with a small piece of zinc. The liquid is poured off from the precipitated copper, which is then washed a few times with water, alcohol, and ether, dried at 100° and weighed.

For the phosphorus determination fuse 1.5 to 1.8 g. with 30 g. of the oxidizing mixture (30–35 cc. of nitric acid).

[1] Maly's Jahrb. f. Thierchemie, 1886, 55.

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VII.

ANALYSIS OF BLOOD.

If the blood has stood for any length of time, it must be thoroughly shaken in order to avoid errors which may arise from the precipitation of the blood-corpuscles. Small quantities of blood cannot be easily measured without rinsing out the pipette, which is not permissible with aqueous solutions, and also causes a small error in the case of blood. Instead of this we may weigh off the necessary quantities, but in this case we must refer the results to 1 kg. of blood (instead of one liter). It is also very advantageous to dilute the blood beforehand. Let 25 cc. of the blood flow into a 100-cc. measuring-flask, rinse the pipette with distilled water, and fill up to the 100-cc. mark. Take out portions, mixing thoroughly by shaking each time before any of the blood is removed.

1. For the determination of the water and ash 5 cc. of the blood or 10 to 20 cc. of the diluted blood is sufficient.

2. For the nitrogen determination heat 5 cc. of the blood with 10 to 15 cc. of sulphuric acid and 0.5 g. of $CuSO_4$ (placing 60 cc. of fifth-normal acid in the receiver), or use 10 cc. of the diluted blood (with 35 cc. of the fifth-normal acid in the receiver), and titrate back with tenth-normal ammonia solution.

3. For the phosphorus determination heat 5 cc. of blood or 20 cc. of the diluted blood with 30 g. of the oxidizing mix-

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ture (30–35 cc. of nitric acid), or oxidize according to the method of A. Neumann, page 215.

4. For the sulphur determination fuse the same quantities with the oxidizing mixture, or oxidize first with nitric acid, as in the case of meat.

5. For the determination of fat 20 to 25 cc. of the blood are weighed off (less may also be used), poured into five times its volume of absolute alcohol contained in a wide-necked glass-stoppered bottle, and repeatedly shaken. (The pipette must not be rinsed here. It is best to fasten it in a clamp and let it stand for some time, when the blood gradually collects and may be blown out. If the blood has been weighed [in a large weighing-glass], then rinse out the glass with alcohol.) Filter next day through a dry filter, wash once with absolute alcohol, and let the filter dry. Evaporate the alcoholic solution to dryness, dissolve the residue in ether, place the ether extract in the Soxhlet flask and the filter with its contents (or the coagulum alone, if it can be separated from the filter without any loss) in the extraction-thimble, and determine the fat in the usual manner.

6. **Determination of Iron.** Evaporate to dryness on the water-bath between 15 and 20 g. (or cc.) of blood in a platinum or porcelain dish and cautiously carbonize until vapors cease to be evolved. The residue is then warmed with dilute hydrochloric acid free from iron, the solution filtered through a filter containing no iron, and the filter washed with water until the filtrate no longer has an acid reaction. The filter with the carbon is dried, and the dish also. The filter is then placed in the dish and ignited until the carbon is completely burned. The hydrochloric acid solution is then poured into the dish, about twenty drops of dilute sulphuric acid are added, the contents of the dish are evaporated to complete dryness on the water-bath and ignited. Cover the residue remaining after ignition with a

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mixture of three volumes of concentrated sulphuric acid and two volumes of water, warm until dissolved, and dilute to 50 to 100 cc. To determine the iron in the solution we make use of the reaction of potassium permanganate on a solution of ferrous sulphate containing free sulphuric acid. This takes place according to the following equation:

$$\underset{\text{Ferrous sulphate}}{10FeSO_4} + \underset{\text{Potassium permanganate}}{2KMnO_4} + \underset{\text{Sulphuric acid}}{9H_2SO_4} =$$

$$\underset{\text{Ferric sulphate}}{5Fe_2(SO_4)_3} + \underset{\text{Manganous sulphate}}{2MnSO_4} + \underset{\text{Monopotassium sulphate}}{2HKSO_4} + 8H_2O.$$

In the solution obtained above, however, the iron is present as a ferric salt. If we wish to determine the iron by titration with potassium permanganate, we must first convert it into a ferrous salt. This may be most readily accomplished by means of some metallic zinc (weighed quantity about 1 g.). The reduction is made in an atmosphere of carbon dioxide and is continued until the fluid has become perfectly colorless and the zinc has completely dissolved (some dilute sulphuric acid must eventually be added). Let the contents of the flask cool completely in the atmosphere of carbon dioxide, and titrate with a solution of potassium permanganate whose strength has been accurately determined. To prepare this solution weigh off accurately 0.32 g. of pure potassium permanganate, dissolve in water, and dilute to the volume of one liter. This solution may be standardized by means of either oxalic acid or ferrous ammonium sulphate.

Standardization with Oxalic Acid. Weigh off accurately 0.63 g. of perfectly pure oxalic acid which has not effloresced, dissolve in water, and dilute to one liter. Add to 25 cc. of this solution a few cubic centimeters of dilute sulphuric acid, heat in a flask on the wire gauze to boiling, and let the permanganate solution run in from a burette

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until the color of the permanganate no longer completely disappears on shaking. The first permanent faint red color indicates the end-point of the reaction. This is best seen when the flask is placed on a sheet of white paper. If exactly 25 cc. are used, then the strength of the solution is correct and each cubic centimeter of the permanganate solution corresponds to 0.56 mg. of iron. If such an exact agreement is not found, it is best not to change the strength of the solution, but to calculate its value. For example, if, instead of 25 cc., 25.6 cc. were used, then the solution is too dilute and 1 cc. does not correspond to 0.56 mg. of iron, but to $\frac{0.56 \times 25}{25.6}$ = 0.5468 mg.

Standardization with Ferrous Ammonium Sulphate. Weigh off exactly 3.924 g. of pure, dry ferrous ammonium sulphate, $FeSO_4,(NH_4)_2SO_4+6H_2O$ (none of the crystals should be yellow-colored), dissolve in water which has previously been boiled, and dilute the solution with boiled and cold water to one liter. Titrate as with the oxalic acid, but at room temperature.

Titration of the Solution Obtained from the Blood. The titration with the permanganate solution is carried out exactly in the same manner as in standardizing with the ferrous ammonium sulphate solution. Since, however, the zinc contains traces of iron, a check experiment is made with the zinc alone. This procedure does not take into account the slight reducing action of the carbon contained in the zinc.[1] The amount of metallic iron in oxyhæmoglobin is approximately 0.4 per cent. The amount of the oxyhæmoglobin is therefore obtained from the amount of iron found by multiplying by $\frac{100}{0.4} = 250$.

[1] To avoid this action it is also recommended to dilute the solution obtained exactly to 100 cc., let settle, and then take out with the pipette 50 cc. for the determination.

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Simplification of the Method of Preparing the Ash. The above method of preparing the ash may be simplified by extracting the carbon with water instead of with hydrochloric acid. The aqueous extract may be disregarded, as it contains no iron,[1] and the ash remaining may be directly dissolved in the sulphuric acid of the concentration given above. The complete oxidation of the ash takes place with somewhat more difficulty.

The preparation of the ash may also be accomplished more conveniently by not evaporating the blood directly and then igniting, but by treating it first according to Kjeldahl. Fifteen to twenty cubic centimeters of blood are heated with 20 to 30 cc. of sulphuric acid and some mercuric oxide or mercuric chloride solution. Ultimately some more hot sulphuric acid is added and the heating is continued until the solution has become colorless. When cold this is placed in a platinum dish, the flask rinsed with a small quantity of water, the excess of sulphuric acid driven off on the sand-bath, and then ignited completely. Although this method may perhaps appear more roundabout, yet it is really more convenient. The method of previously heating with sulphuric acid in a Kjeldahl flask may also be advantageously used for the quantitative determination of many other ash constituents, as well as for the detection of metallic poisons.

The destruction of the organic matter may be more readily accomplished according to the method of A. Neumann by using with the sulphuric acid the same amount of ammonium nitrate.

Gravimetric Determination of the Iron. If only single determinations of iron are to be made, instead of a long series, the gravimetric determination as ferric phosphate, $FePO_4$, is more convenient. For this purpose add to the

[1] It may, however, contain iron if the extract is highly colored.

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solution obtained from the ash and containing all of the iron as ferric salt a few cubic centimeters of sodium phosphate solution, make alkaline with ammonia, and acidify with acetic acid. The precipitate of ferric phosphate resulting is collected on an ash-free filter, washed, dried, ignited (in a porcelain crucible), and weighed. One hundred parts correspond to 52.98 parts of Fe_2O_3 or to 37.09 parts of Fe.

Since by this method it makes no difference if the solution still contains traces of organic matter, the evaporation of the diluted sulphuric acid, ignition, and dissolving again in sulphuric acid (see above) may be omitted. (In the titration with permanganate this procedure is also frequently unnecessary). Frequently also all the iron passes so completely into the hydrochloric acid solution (especially in the determination of iron in the organs, which is made in an exactly analogous manner) that it is sufficient to treat the ash remaining with a little hydrochloric acid, filter the diluted solution, add it to the first hydrochloric acid extract, and then precipitate the iron directly from the hydrochloric acid solution. Hydrochloric acid solutions cannot, however, be titrated with potassium permanganate solution.

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VIII.

DETERMINATION OF HYDROCHLORIC ACID IN THE GASTRIC JUICE ACCORDING TO SJÖQVIST.

Twenty-five cubic centimeters of the hydrochloric acid solution A (see page 33) and the same amount of the lactic acid solution are mixed in a dry beaker. Ten cubic centimeters of this mixture are then measured off with a pipette, placed in an evaporating-dish, a few drops of albumose solution and a small quantity of absolutely pure barium carbonate [1] added, and the mixture heated on the water-bath. During the heating the mixture is thoroughly stirred with a thin glass rod. This is then rinsed off and the mixture evaporated to dryness. The dry residue now consists of barium chloride, barium lactate, excess of barium carbonate, albumose, and the salts which may be present and which are never absent from the fluids of the stomach. This is now heated over a free flame and ignited gently until most of the carbon is consumed (complete oxidation is superfluous). By this means the organic substances are burnt up or carbonized, the barium lactate is converted into barium carbonate, while the barium chloride remains unchanged. Let the dish cool, extract the residue with hot water, and filter through a small filter of thin paper (for example, Schleicher and Schüll's No. 590), of at most 9 cm. diameter.

[1] If the barium carbonate contains alkali carbonate, which is very frequently the case, the amount of hydrochloric acid found will be too low.

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Only barium chloride, together with some salts present in solution, passes through the filter, while the insoluble barium carbonate remains behind. Wash the dish with hot water, pour into the filter which was previously used, and wash until all the barium chloride has been completely washed out of the filter. By working carefully this may be accomplished without increasing the volume of the filtrate and the wash-water to more than 50 to 60 cc. We may also continue the washing somewhat longer and then cautiously evaporate to 50 cc. In any case the last wash-water which is thought to be free from barium chloride is collected by itself and tested with silver nitrate and nitric acid.[1] **The filtrate and the wash-water contain all the hydrochloric acid of the gastric juice combined with barium. The amount of barium is hence a direct measure of the amount of hydrochloric acid.**

To determine the amount of barium, acidify the filtrate and wash-water with a few drops of hydrochloric acid, heat in a beaker on the wire gauze until boiling begins, add about 4 to 5 cc. of dilute sulphuric acid (also previously heated), then heat further on the water-bath until the barium sulphate settles, leaving a clear solution. Filter through a close, ash-free filter[2] of about 9 cm. diameter, transfer the precipitate completely to the filter—the filtrate must be perfectly clear; if it is not, it must be again passed through the same filter—and wash until a portion of the wash-water is no longer rendered turbid by silver nitrate solution or by barium chloride solution. Then fill the filter once with

[1] The original direction, to wash until a portion of the wash-water is no longer made turbid with sulphuric acid, is not to be adhered to, since such a point, in consequence of the slight solubility of the barium carbonate in water, is not to be attained. Too long-continued washing may therefore also lead to a plus error.

[2] Suitable filter-papers are the No. 590 of Schleicher and Schüll, and the ash-free baryta filter-papers Nos. 400 and 412 of Dreverhoff in Dresden.

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absolute alsohol and once with ether, let the excess of ether evaporate, place the filter together with the barium sulphate in a weighed platinum crucible, heat, at first gently and then more strongly, with the crucible half open, until all the carbon is burned, and weigh when cold (see page 206). One molecule of barium sulphate, $BaSO_4$, corresponds to two molecules of hydrochloric acid, HCl; 233.46 parts by weight correspond to 72.92 parts of hydrochloric acid, HCl. The entire determination should be made in duplicate as a check.

Instead of precipitating the barium as sulphate and weighing we may also proceed as follows: Add to the aqueous solution of barium chloride obtained ammonia and ammonium carbonate, filter off the precipitated barium carbonate, wash thoroughly, and dissolve in dilute hydrochloric acid. For this purpose it is best to wash the barium carbonate into a beaker, dissolve it in dilute hydrochloric acid, filter the solution through the filter which was used to collect the barium carbonate, and wash. The solution is evaporated to complete dryness on the water-bath to remove any hydrochloric acid still present, a few cubic centimeters of water are added, and the solution is again evaporated to dryness. The residue is dissolved in water and titrated with a dilute solution of silver nitrate of known strength, after the addition of sufficient potassium chromate solution.[1] A solution of silver nitrate containing 2.9054 g. of $AgNO_3$ to the liter, 1 cc. of which equals 0.001 g. NaCl, should be used. If this silver solution is used the percentage amount of HCl in the gastric juice may be calculated from the formula $x = \frac{n \times 3.646}{585}$, in which n indicates the number of cubic centimeters of the silver solution used for 10 cc. of the gastric juice.

[1] The amount of the potassium chromate solution added must not be too small, as the potassium chromate reacts with the barium chloride to form insoluble barium chromate and potassium chloride.

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IX.

QUANTITATIVE DIGESTION EXPERIMENTS.

For a short series of experiments fresh fibrin and coagulated egg-albumen may be used; for a longer series only material containing a fixed amount of water can be used. For example, fibrin which has been treated with alcohol and ether and then powdered, coagulated egg-albumen treated with alcohol and ether, etc. In any case, care must be taken that, in any series of experiments, the same material is always used. This should be prepared beforehand in large quantities, and it must be kept in closed vessels, since the amount of water which it contains must not change. Blood-serum which has been preserved by the addition of chloroform may also be used.[1] The chloroform must be expelled by means of an air-current before using the serum. The results are not quite equivalent, but depend upon the nature of the material used. Slight disturbing influences do not often appear when fresh fibrin is used, but only when hard-boiled egg-albumen is used. Furthermore, weak disturbing influences are sometimes more perceptible when pepsin-hydrochloric acid is used than when the extract of the lining of the stomach is made use of. Finally, the duration of the digestion is also of influence. It is often neces-

[1] Fluid egg-albumen can no longer be used, since we have learned that it contains ovomucoid, which necessarily causes an error when we base our opinion regarding the digestibility on the quantity of the portion dissolved during the digestion.

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sary, in order to recognize disturbing influences, to shorten the duration of the digestion even to four hours. The answer to the question whether a substance disturbs the digestion cannot, therefore, strictly speaking, be given in general, but only applies for the special conditions adhered to in the experiment. Of course there are also substances which interfere with the digestion even when the conditions for the digestion are the most favorable, e.g., larger quantities of sugar, gum, or plant-mucus.[1] When fibrin or coagulated albumin, etc., is used we may either determine the undissolved albumin or the dissolved or both. When an albumin solution is used the determination of that which remains undissolved is of course omitted. If we limit ourselves to the determination of the portion remaining undissolved, then the precipitate formed on neutralization would be included among the products of the digestion, which is scarcely justifiable. At all events, it is preferable to determine the undissolved albumin + the precipitable albumin on the one hand and the peptonized (albumose and peptone) on the other, or to determine the quantity of the albumin present at the beginning of the digestion and the quantity of the peptonized material in the mixture of digestion products.[2] It is advisable to replace the very long and also not quite accurate determination of the dry residue by the Kjeldahl nitrogen determination. One example of this kind of experiment may suffice.

Blood-serum is treated with an equal amount of water, shaken thoroughly, and exactly neutralized with dilute hydrochloric acid.

[1] Mugdan, Berl. klin. Wochenschr., 1891, No. 32.

[2] If we also estimate at the same time the quantity of the coagulable albumin (including the residue remaining undissolved) in the mixture of the digestion products, then we have a check on the correctness of the analysis: the sum of these values and the albumoses must equal the quantity of the albumin used.

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Place in each of a series of flasks or bottles (with stoppers) 50 cc. of pepsin-hydrochloric acid. In each of another series place 50 cc. of the pepsin-hydrochloric acid which contains the weighed quantity of the material whose influence is to be tested, or add to a number of portions of the pepsin-hydrochloric acid the material to be tested and dissolve it by shaking without warming. Then place in each flask 20 cc. of the albumin solution, shake thoroughly, and digest it at 40°, shaking repeatedly during the digestion. To avoid accidental errors, each mixture must be prepared in duplicate.

To determine the amount of nitrogen in the albumin solution, heat 20 cc. of the solution with 15 cc. of concentrated sulphuric acid, 10 g. of potassium sulphate, and 0.5 g. of copper sulphate. This determination is also to be made in duplicate. The heating must be done at first with great care, otherwise the determination may be lost from foaming. When the heating has continued about one and one-half hours and the oxidation is not then completed, it is advisable to let cool, add 10 to 15 cc. of sulphuric acid and heat again. When the oxidation is completed, which may be accomplished even without the addition of potassium permanganate, let cool, dilute the solution, let cool again, put it in a measuring-flask and fill up to 100 cc. Twenty-five or fifty cubic centimeters of the well-mixed solution are used for the determination of the ammonia or amount of nitrogen. To collect the ammonia 20 to 40 cc. of fifth-normal hydrochloric acid are sufficient. The amount of albumin is obtained by multiplying the amount of nitrogen found by 6.25.

After the digestion of the albumin has continued the desired number of hours, neutralize the mixture with dilute caustic soda solution (half- or fourth-normal), heat to boiling, add acetic acid to faintly acid reaction and 5 cc. of concentrated

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sodium chloride solution in order to completely precipitate all the albumin. Then let cool, place the entire fluid together with the precipitate in a 100- or 200-cc. measuring-flask, rinse the vessel thoroughly, fill up to the mark, filter through a dry filter, and take a quarter or half of the whole amount for the nitrogen determination.

The amount of nitrogen contained in the pepsin-hydrochloric acid is so small that it may be neglected. It must be considered, however, when an extract of the mucus membrane of the stomach is used. Of course the amount of nitrogen in the substance tested is also to be taken into consideration.

It is not always possible to proceed as above; quite often the method must be modified. For the arrangement of these experiments see Virchow's Arch. **120**, 353; **122**, 238; **127**, 514; Berl. klin. Wochenschr. 1891, No. 32; Virchow's Arch. **150**, 260 (1897)..

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X.

QUANTITATIVE DETERMINATION OF GLYCOGEN.[1]

Place 100 g. of the ground meat or organ and 100 cc. of 60 per cent. potassium hydroxide solution in a 200-cc. flask. Use the purest potassium hydroxide made by "Merck." The strength of the potassium hydroxide solution is determined accurately with standard hydrochloric acid.

Before placing the flask in the boiling water-bath shake it once or twice in order to mix the contents thoroughly, and repeat the shaking after the flask has been heated one-quarter to one-half of an hour. As soon as the liquid has assumed the temperature of the boiling water-bath and no further expansion takes place, close the flask with a rubber stopper.

After heating for two hours, take the flask out of the boiling water-bath, empty it into a 400-cc. measuring-flask, and rinse it out with boiling water. When the liquid is cold fill the 400-cc. flask up to the mark with sterilized water and filter the solution through glass wool until the filtrate is quite clear or only slightly opalescent. From a burette measure off 100 cc. of the filtered meat solution into a 300-cc. beaker, add 100 cc. of 96 per cent. (Tralles) alcohol, and mix thoroughly with a glass rod. The precipitate of glycogen settles very rapidly, so that it may be filtered off even after a quarter of an hour. It is safer, however, to wait some hours or,

[1] Pflüger in Pflüger's Arch. 93, 163.

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best, overnight. Filter through a 15-cm. filter (Munktell) and wash the precipitate with a mixture of one volume of 15 per cent. potassium hydroxide solution and two volumes of alcohol (96% Tr.). Collect this wash-fluid in the beaker in which the glycogen was precipitated and pour it through the filter twice. Then wash the glycogen on the filter with 96 per cent. alcohol.

After the alcohol has drained thoroughly draw a well-cleaned rubber tube over the end of the funnel-tube and close it with a pinch-cock. Under the rubber tube place the empty beaker in which the precipitation of glycogen took place. It makes no difference if a little glycogen sticks to the walls of the beaker. Then fill the funnel full of sterilized but cold water. After one-half to one hour the glycogen will have almost completely dissolved. Open the pinch-cock and let the solution run into the beaker. Then close the rubber tube again, pour some more water into the funnel, wait till all the glycogen has dissolved, and again open the pinch-cock. After the water has run out a second time close the pinch-cock again, fill the filter half full of water, and wash the fine green dust from the paper with the aid of a fine brush. This dust is insoluble in water. Finally allow the wash-water to flow into the beaker. Then place a small piece of litmus paper in the filtrate and let hydrochloric acid, 1.19 specific gravity, run in from a burette, drop by drop, mixing thoroughly with a glass rod until the alkali is exactly neutralized.

Then place a funnel in a 500-cc. measuring-flask and pour in the neutralized glycogen solution with all the necessary precautions observed in quantitative analysis.

Measure out of the burette 25 cc. of hydrochloric acid of 1.19 specific gravity into the beaker and pour this also into the 500-cc. flask. Then place the flask with its funnel under the exit-tube of the funnel which contains the glycogen filter. Fill the beaker with water and pour it on the filter

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in order to wash out the last traces of the glycogen, and add it to the 500-cc. flask. Repeat this rinsing until the 500-cc. flask is almost but not quite filled to the mark. Finally allow a few cubic centimeters of the fluid to run into a test-tube and add several cubic centimeters of 96 per cent. alcohol. No turbidity should result. Then close the 500-cc. flask with a rubber stopper and shake the mixture thoroughly. It contains approximately 2.2 per cent. hydrochloric acid.

These directions must be modified:

1. When such a small quantity of glycogen is present that an exact analysis cannot be carried out. In this case we precipitate the glycogen with an equal volume of alcohol, not from 100 cc. of the filtered meat solution, but from 200 or 300 cc., and proceed as directed above.

2. When the glycogen is to be estimated in a very small organ, e.g., in the liver of a chicken. In this case weigh off 10 g. of the finely chopped liver and put it into a small flask with 10 cc. of 60 per cent. potash solution. After heating for two hours in the water-bath, cool and fill up to 40 cc. with water. Filter through glass wool and use 25 to 30 cc. for precipitation with an equal volume of 96 per cent. alcohol. Filter through a small Swedish filter and proceed as directed above.

The solution of the glycogen on the filter must be made so that not more than 100 cc. of the filtrate with 2.2 per cent. of hydrochloric acid is obtained in a 100-cc. flask.

Determination of the Glycogen by Conversion into Glucose.

The flask containing the glycogen solution is placed in a boiling-water-bath and closed with a rubber cork as soon as the expansion of the liquid caused by the heat has ceased. After heating for three hours, remove the flask from the bath, let it cool, and fill up to the mark with water. Then filter the sugar solution through a dry Swedish filter into a

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500-cc. flask. The glucose in this solution is then determined gravimetrically (see Determination of Glucose, page 200).

According to Salkowski [1] it is much better not to treat the fresh liver with caustic potash solution, but to subject it to a preliminary treatment with alcohol and ether. This is done by extracting the finely chopped liver with absolute alcohol and then with ether, thus converting it into a fine powder. This dissolves comparatively readily in 2–3 per cent. potassium hydroxide solution, the rapidity of the solution depending upon the completeness of the pulverization of the liver. If this has been well done, then the solution in the caustic potash takes place in a few minutes. The solution is brown-colored and not quite clear owing to the presence of undissolved calcium phosphate. Only comparatively small quantities of the liver powder, 5 to 10 g., need to be taken for the glycogen determination.

The alkaline solution is clarified by letting it stand—it is advisable to take only an aliquot part, in order to avoid filtering, about four-fifths—and then it is precipitated with double its volume of alcohol, the precipitate washed, first with 66 per cent. alcohol, then with stronger alcohol, and dried at 105°. The glycogen thus obtained contains only a trace of nitrogen, though it leaves considerable ash when ignited. It may be determined as glucose after hydrolyzing according to Pflüger's method given above.

The liver powder, prepared as stated above, also dissolves very quickly in artificial gastric juice. With 5 g. of the powder Salkowski found no glycogen in the residue left after digesting for forty-six hours, nor did the coagulum resulting from boiling the solution, after it had been nearly neutralized, contain even a trace of glycogen. From the filtrate, after evaporating it to about 150 cc., the addition

[1] Zeit. f. physiologische Chemie, 36, 257.

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of double the volume of alcohol precipitated glycogen, containing far less ash than that which was obtained from the alkaline solution. This glycogen contained only traces of nitrogen, and its solution gave no precipitate with Brücke's reagent and hydrochloric acid.

A simple method depending on the auto-digestion of the liver is described by Austin, Virchow's Arch. 150, 185 (1897). It yields approximately correct results.

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APPENDIX I.

REAGENTS.

The reagents mentioned in the text, unless otherwise stated, are solutions of the following concentration or of substances which must show the following standard of purity:

Acetic Acid, containing 30 per cent. of acetic acid.

Alcohol. Must be colorless and leave no residue on evaporation.

Alkaline Barium Chloride Solution. Two volumes of baryta-water and one volume of barium chloride solution.

Almèn's Solution. Dissolve 4 g. of tannin in 8 cc. of 25 per cent. acetic acid and then add 190 cc. of 40 to 50 per cent. alcohol.

Ammonia. A solution of ammonia in water, specific gravity 0.96, containing about 10 per cent. NH_3.

Ammonium Carbonate. One part [1] of the commercial ammonium carbonate, one part of ammonia, and four parts of water. Must stand some days before being used.

Ammonium Chloride, 1:10.[2]

Ammonium Molybdate in acid solution. Dissolve 50 g. of molybdic acid in 200 g. of 8–10 per cent. ammonia and pour the solution into 750 g. of nitric acid, specific gravity 1.2. Let stand for some days in a warm place and then decant.

Ammonium Oxalate, 1:25.

[1] By parts is always meant parts by weight.

[2] Distilled water is always to be used as the solvent; 1:10 means 1 part by weight of ammonium chloride dissolved in 10 parts by weight of water. The water, of course, is to be measured.

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Barium Carbonate, precipitated from barium chloride solution by ammonium carbonate and well washed.

Barium Chloride, 1:10.

Barium Nitrate, 1:12.

Baryta-water. One part of crystallized caustic baryta heated with fifteen parts of water and, after cooling, filtered.

Basic Lead Acetate. Lead subacetate solution, commercial. (Liquor Plumbi Acetici Ph. G. IV.)

Bromine-water. Water shaken with an excess of bromine and allowed to stand till the bromine has settled.

Brücke's Reagent (potassium mercuric iodide solution). Heat a solution of potassium iodide containing 100 g. of KI in the liter, and add mercuric iodide as long as it dissolves. After cooling decant from the red crystals and add a few crystals of potassium iodide.

Calcium Chloride. Dry pure calcium chloride, 1:10.

Cochineal Tincture. Five grams of cochineal, 150 cc. of alcohol, and 100 cc. of water are allowed to stand for some days at room temperature; the solution is then poured off and filtered. The cochineal remaining may be used over again.

Copper Sulphate, 1:10.

Ether. Must leave, on evaporation, no residue having an odor or an acid reaction.

Ferric Chloride, 3:100.

Hydrochloric Acid, specific gravity 1.183.

Lead Acetate, 1:10.

Magnesia Mixture. One part of crystallized magnesium sulphate, two parts of ammonium chloride, four parts of ammonia, and eight of water, or 110 g. of magnesium chloride (chemically pure), 140 g. of ammonium chloride, 700 cc. of 8 per cent. ammonia, and 1300 parts of water (or 250 cc. of ammonia, specific gravity 0.91, and 1750 cc. of water). Let stand for some days before using.

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Magnesium Sulphate, cold, saturated solution.

Mercuric Chloride, 1:20.

Millon's Reagent. Warm one part of mercury with two parts of nitric acid, specific gravity 1.4, until the mercury is completely dissolved. Dilute one volume of the solution with two volumes of water.

O. Nasse (Arch. f. d. ges. Physiol. 83, 361 (1901).) recommends an aqueous solution of mercuric acetate, to which, just before using, a few drops of a 1 per cent. potassium nitrite solution are to be added and also, in case the reaction is not distinctly acid, a little dilute acetic acid.

Nessler's Reagent. Dissolve 50 g. of potassium iodide in the same quantity of water, then heat and add a hot, concentrated mercuric chloride solution until some mercuric iodide remains undissolved. (Twenty to twenty-five grams of $HgCl_2$ are required.) Filter and add 150 g. of potassium hydroxide dissolved in 300 g. of water, then dilute to one liter, add 5 cc. more of the mercuric chloride solution, let the precipitate settle and decant. The solution is kept in small closed bottles. It must be completely saturated with mercuric iodide, otherwise the reagent is not very sensitive.

Nitric Acid. Must be free from hydrochloric acid and colorless, and free from nitrous acid, specific gravity 1.2.

Nylander's Solution. One hundred grams of caustic soda solution of 1.119 specific gravity (10.33 g. NaOH), 4 g. of potassium sodium tartrate, and 2 g. of bismuth subnitrate.

Oxidizing Mixture. Three parts of KNO_3 and one part of Na_2CO_3, dry and pure.

Phosphotungstic Acid, 1:20, acidified with hydrochloric acid.

Platinum Chloride. Must dissolve and give a clear solution in alcohol, 1:10.

Potassium Chromate. Yellow chromate of potassium, K_2CrO_4, 1:20.

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Potassium Ferrocyanide, 1:10.

Potassium Hydroxide. One part of potassium hydroxide (pure, fused) to two parts of water.

Potassium Nitrate. Must be free from chlorides and sulphates.

Potassium Sulphocyanate, 1:20.

Rosolic Acid, one part in 100 to 200 parts of alcohol.

Silver Nitrate, 1:50.

Sodium Carbonate. Dehydrated and a saturated solution, must be free from chlorides and sulphates.

Sodium Chloride. Cold saturated solution (water shaken for some time with an excess of powdered sodium chloride). One hundred cubic centimeters of the solution contain **31.84 g.** of sodium chloride.

Sodium Hydroxide, containing about 15 g. of sodium hydroxide in 100 g. of the solution, specific gravity 1.17.

Sodium Phosphate, Na_2HPO_4, 1:10.

Sulphuric Acid. By this term is always meant, unless otherwise stated, an acid containing 200 g. of concentrated sulphuric acid in the liter.

Tartaric Acid, pulverized.

Turmeric-paper. Digest powdered turmeric-root with alcohol, dip bibulous paper in the solution, and dry in the air.

Zinc Chloride Solution, alcoholic. Thick, sirupy, aqueous solution diluted with alcohol until the specific gravity is 1.2.

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APPENDIX II.

TABLES OF THE SPECIFIC GRAVITIES OF SOME SOLUTIONS.

1. CAUSTIC SODA.

Specific Gravity at 15°.	100 Grams contain NaOH in Grams.	1 Liter contains NaOH in Grams.	Specific Gravity at 15°.	100 Grams contain NaOH in Grams.	1 Liter contains NaOH in Grams.
1.007	0.61	6	1.190	16.77	200
1.014	1.20	12	1.200	17.67	212
1.022	2.00	21	1.210	18.58	225
1.029	2.71	28	1.220	19.58	239
1.036	3.35	35	1.231	20.59	253
1.045	4.00	42	1.241	21.42	266
1.052	4.64	49	1.252	22.64	283
1.060	5.29	56	1.263	23.67	299
1.067	5.87	63	1.274	24.81	316
1.075	6.55	70	1.285	25.80	332
1.083	7.31	79	1.297	26.83	348
1.091	8.00	87	1.308	27.80	364
1.100	8.68	95	1.320	28.83	381
1.108	9.42	104	1.332	29.93	399
1.116	10.06	112	1.345	31.22	420
1.125	10.97	123	1.357	32.47	441
1.134	11.84	134	1.370	33.69	462
1.142	12.64	144	1.383	34.96	483
1.152	13.55	156	1.397	36.25	506
1.162	14.37	167	1.410	37.47	528
1.171	15.13	177	1.424	38.80	553
1.180	15.91	188	1.438	39.99	575

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2. CAUSTIC POTASH.

Specific Gravity at 15°.	100 Grams contain KOH in Grams.	1 Liter contains KOH in Grams.	Specific Gravity at 15°.	100 Grams contain KOH in Grams.	1 Liter contains KOH in Grams.
1.007	0.9	9	1.190	21.4	255
1.014	1.7	17	1.200	22.4	269
1.022	2.6	26	1.210	23.3	282
1.029	3.5	36	1.220	24.2	295
1.037	4.5	46	1.231	25.1	309
1.045	5.6	58	1.241	26.1	324
1.052	6.4	67	1.252	27.0	338
1.060	7.4	78	1.263	28.0	353
1.067	8.2	88	1.274	28.9	368
1.075	9.2	99	1.297	30.7	398
1.083	10.1	109	1.320	32.7	432
1.091	10.9	119	1.345	34.9	469
1.100	12.0	132	1.370	36.9	506
1.108	12.9	143	1.397	38.9	543
1.116	13.8	153	1.424	40.9	582
1.125	14.8	167	1.453	43.4	631
1.134	15.7	178	1.483	45.8	679
1.142	16.5	188	1.530	49.4	756
1.152	17.6	203	1.580	53.2	840
1.162	18.6	216	1.615	55.9	902
1.171	19.5	228	1.634	57.5	940
1.180	20.5	242			

3. AMMONIA.

Specific Gravity at 15°.	Per Cent. NH_3.	1 Liter contains NH_3 in Grams.	Specific Gravity at 15°.	Per Cent. NH_3.	1 Liter contains NH_3 in Grams.
0.970	7.31	70.9	0.942	15.04	141.7
0.968	7.82	75.7	0.940	15.63	146.9
0.966	8.33	80.5	0.938	16.22	152.1
0.964	8.84	85.2	0.936	16.82	157.4
0.962	9.35	89.9	0.934	17.42	162.7
0.960	9.91	95.1	0.932	18.03	168.1
0.958	10.47	100.3	0.930	18.64	173.4
0.956	11.03	105.4	0.928	19.25	178.6
0.954	11.60	110.7	0.926	19.87	184.2
0.952	12.17	115.9	0.920	21.75	200.1
0.950	12.74	121.0	0.910	24.99	227.4
0.948	13.31	126.2	0.906	26.31	238.3
0.946	13.88	131.3	0.902	27.65	249.4
0.944	14.46	136.5	0.900	28.33	255

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4. HYDROCHLORIC ACID.

Specific Gravity $d\frac{15°}{4°}$.	100 Grams contain HCl in Grams.	1 Liter contains HCl in Kilograms.	Specific Gravity $d\frac{15°}{4°}$.	100 Grams contain HCl in Grams.	1 Liter contains HCl in Kilograms.
1.200	39.11	0.469	1.115	22.86	0.255
1.195	38.16	0.456	1.110	21.92	0.243
1.190	37.23	0.443	1.105	20.97	0.232
1.185	36.31	0.430	1.100	20.01	0.220
1.180	35.39	0.418	1.095	19.06	0.209
1.175	34.42	0.404	1.090	18.11	0.197
1.171	33.65	0.394	1.085	17.13	0.186
1.170	33.46	0.392	1.080	16.15	0.174
1.165	32.49	0.379	1.075	15.16	0.163
1.163	32.10	0.373	1.070	14.17	0.152
1.160	31.52	0.366	1.065	13.19	0.141
1.155	30.55	0.353	1.060	12.19	0.129
1.152	29.95	0.345	1.055	11.18	0.118
1.150	29.57	0.340	1.050	10.17	0.107
1.145	28.61	0.328	1.045	9.16	0.096
1.1425	28.14	0.322	1.040	8.16	0.085
1.140	27.66	0.315	1.035	7.15	0.074
1.135	26.70	0.303	1.030	6.15	0.064
1.130	25.75	0.291	1.025	5.15	0.053
1.125	24.78	0.278	1.020	4.13	0.042
1.120	23.82	0.267	1.015	3.12	0.032

5. NITRIC ACID.

Specific Gravity $d\frac{15°}{4°}$.	100 Grams contain HNO_3 in Grams.	1 Liter contains HNO_3 in Kilograms.	Specific Gravity $d\frac{15°}{4°}$.	100 Grams contain HNO_3 in Grams.	1 Liter contains HNO_3 in Kilograms.
1.520	99.67	1.515	1.360	57.57	0.783
1.509	97.84	1.476	1.345	54.93	0.739
1.495	91.60	1.369	1.335	53.22	0.710
1.485	87.70	1.302	1.315	49.89	0.656
1.475	84.45	1.246	1.295	46.72	0.605
1.465	81.42	1.193	1.275	43.64	0.556
1.455	78.60	1.144	1.250	39.82	0.498
1.440	74.68	1.075	1.225	36.03	0.441
1.430	72.17	1.032	1.195	31.62	0.378
1.420	69.80	0.991	1.185	30.13	0.357
1.405	66.40	0.933	1.175	28.63	0.336
1.395	64.25	0.896	1.165	27.12	0.316
1.375	60.30	0.829	1.155	25.60	0.296

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6. ALCOHOL. 60° F. = 15.56° C.

$d\frac{15.56°}{15.56°}$	Volume, Per Cent.	Weight Per Cent.	$d\frac{15.56°}{15.56°}$	Volume, Per Cent.	Weight Per Cent.
0.89499	68	60.48	0.84961	85	79.58
0.89256	69	61.53	0.84660	86	80.80
0.89010	70	62.59	0.84355	87	82.03
0.88762	71	63.66	0.84044	88	83.28
0.88511	72	64.74	0.83726	89	84.54
0.88257	73	65.83	0.83400	90	85.82
0.88000	74	66.92	0.83065	91	87.12
0.87740	75	68.02	0.82721	92	88.44
0.87477	76	69.13	0.82365	93	89.79
0.87211	77	70.26	0.81997	94	91.16
0.86943	78	71.39	0.81616	95	92.56
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		O = 16
Aluminium	Al	27.1
Antimony	Sb	120.2
Argon	A	39.9
Arsenic	As	75
Barium	Ba	137.4
Beryllium	Be	9.1
Bismuth	Bi	208.5
Boron	B	11
Bromine	Br	79.96
Cadmium	Cd	112.4
Cæsium	Cs	133
Calcium	Ca	40.1
Carbon	C	12
Cerium	Ce	140
Chlorine	Cl	35.45
Chromium	Cr	52.1
Cobalt	Co	59
Copper	Cu	63.6
Erbium	Er	166
Fluorine	F	19
Gadolinium	Gd	156
Gallium	Ga	70
Germanium	Ge	72.5
Gold	Au	197.2
Helium	He	4
Hydrogen	H	1.008
Indium	In	114
Iodine	I	126.85
Iridium	Ir	193
Iron	Fe	55.9
Krypton	Kr	81.8
Lanthanum	La	138.9
Lead	Pb	206.9
Lithium	Li	7.03
Magnesium	Mg	24.36
Manganese	Mn	55
Mercury	Hg	200
Molybdenum	Mo	96
Neodymium	Nd	143.6
Neon	Ne	20
Nickel	Ni	58.7
Niobium	Nb	94
Nitrogen	N	14.04
Osmium	Os	191
Oxygen	O	16
Palladium	Pd	106.5
Phosphorus	P	31
Platinum	Pt	194.8
Potassium	K	39.15
Praseodymium	Pr	140.5
Radium	Ra	225
Rhodium	Rh	103
Rubidium	Rb	85.4
Ruthenium	Ru	101.7
Samarium	Sa	150
Scandium	Sc	44.1
Selenium	Se	79.2
Silicon	Si	28.4
Silver	Ag	107.93
Sodium	Na	23.05
Strontium	Sr	87.6
Sulphur	S	32.06
Tantalum	Ta	183
Tellurium	Te	127.6
Terbium	Tb	160
Thallium	Tl	204.1
Thorium	Th	232.5
Thulium	Tu	171
Tin	Sn	119
Titanium	Ti	48.1
Tungsten	W	184
Uranium	U	238.5
Vanadium	V	51.2
Xenon	X	128
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Zinc	Zn	65.4
Zirconium	Zr	90.6

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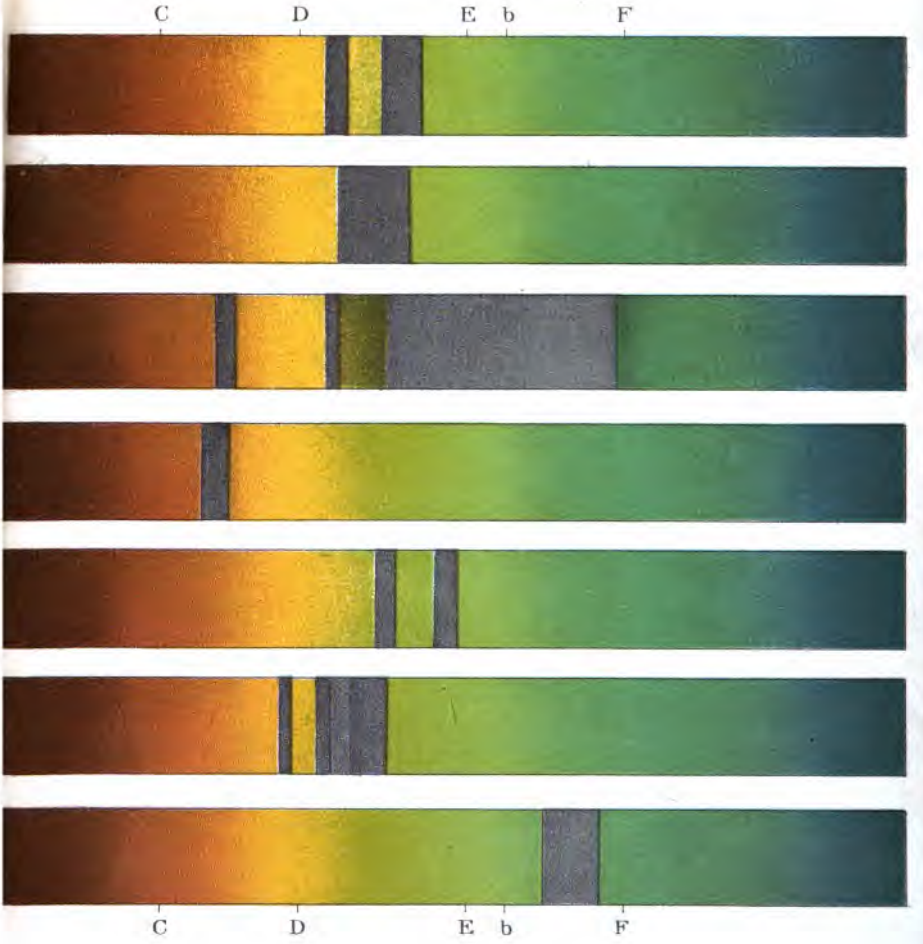
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No. 1. Oxyhæmoglobin.
No. 2. Hæmoglobin.
No. 3. Methæmoglobin (in neutral, or faintly alkaline, solution).
No. 4. Hæmatin in acid alcohol solution.
No. 5. Reduced hæmatin in alkaline solution.
No. 6. Hæmatoporphyrin in acid solution.
No. 7. Urobilin.

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